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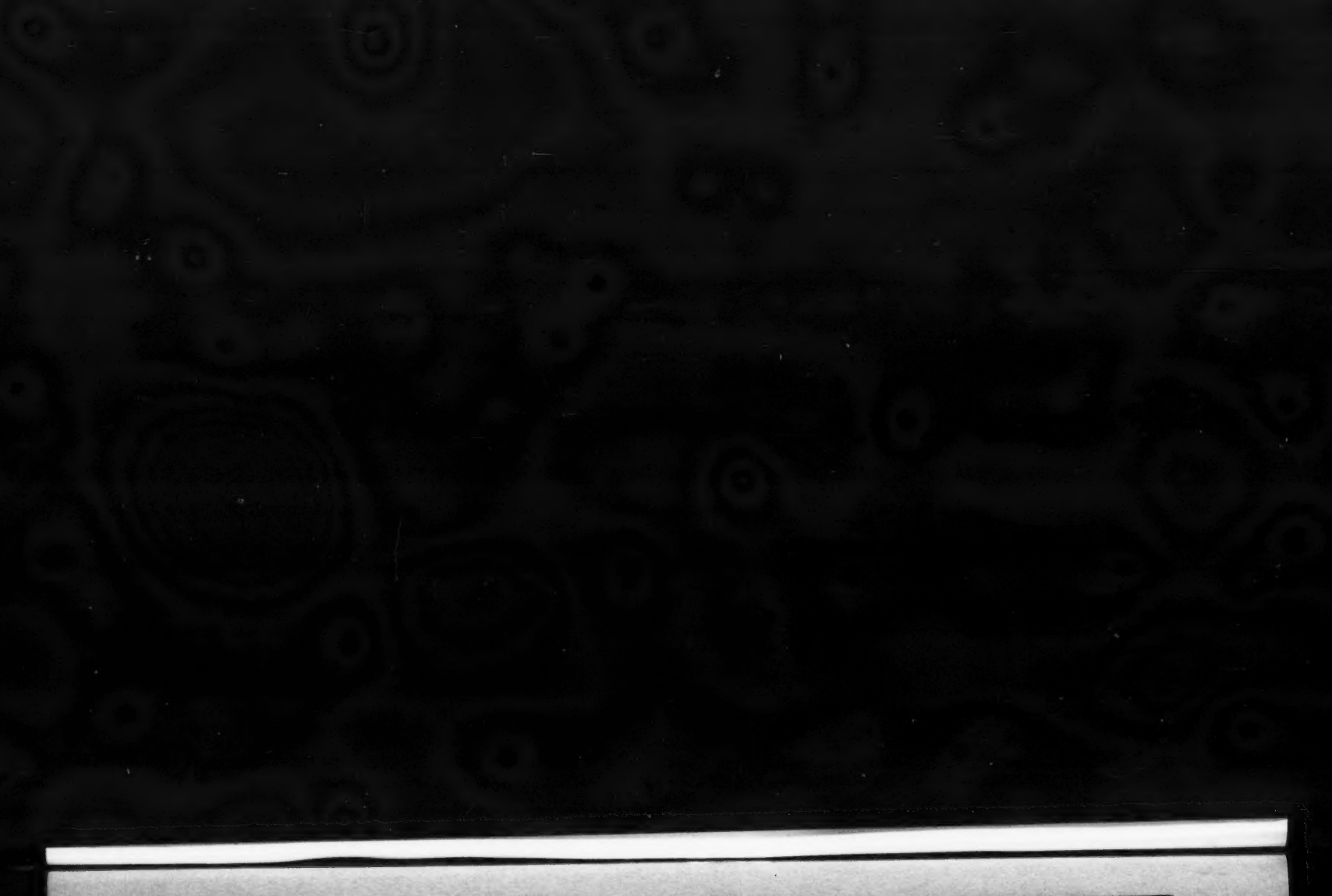

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UNITS AND SYSTEMS

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A. INTRODUCTION

That which follows represents certain portions of a series of lectures given at the Mountain Lake Biological Station of the University of Virginia in the summer of 1938. The material has been prepared for publication at the request of the biologists who attended the seminar where the future course of biological study was discussed. Although the consideration wanders far afield into theory, it grew out of practical efforts to understand the relation between structural conditions in the germ cells and character inheritance. In modern terms this means the relation between specific factors—genes—located within the chromosomes, and the behavior of characters in Mendelian inheritance. Genetical studies show with increasing clearness that, while there are definite connections between presumably discrete factors and recognizable body characters, these are incidental to the operation of the whole system. It seems clear now that every gene is involved in the development of every body character and that their effects differ at each stage of differentiation. There is no longer any thought of a simple, direct influence of a gene upon a single character. It appears increasingly clear that time is an element in the problem. If a factor is prevented from operating to produce a characteristic effect when it should, the whole operation is affected.

There is some evidence also to indicate that if the factor is displaced from its normal position in the series its effect is altered. It has long been thought that the material form of chromosome organization is significant of the manner of its action. The linear structure of chromosomes and their exact meristic division was indeed the ground upon which Roux and Weismann based their identification of the chromosomes with the hereditary determining mechanism. As is stated later in this discussion, it is believed that the chromosomes are built up by successive additions of components in a linear series and that specific functions are exercised by these in the order of their ac-

cession. Because of known translocations, inversions, etc., it is evident that such a spatial-causal relation cannot be viewed too narrowly. At the same time it may well be questioned how persistent in nature are such experimentally produced changes.

It has been demonstrated that in the germ cells there is a particulate organization which stands in a causal relation to the characters of the body. These determinative elements are individually different, but produce specific effects by modifying the coördinated functioning of the entire series. They are located in the chromosomes, and the linked groups of characters correspond in number and size to the paired chromosomes within the cells. The number of characters present in different species,—therefore the number of genetic controls,—must vary accordingly. The effects of these controls change during individual development, producing successively more and more complex results. The order of the appearance of these characters follows a pattern established by successive and repeated reactions between a continuing form of organization and a slowly changing environment. This pattern is not a rigid, formal design, located outside the operating elements in the system, but is in reality the actual order of the incidence of the reactions involved. While for practical purposes we must study intensively the most fruitful aspect of a subject, it is always to be remembered that a full understanding waits upon the correlation of all phases of a study. In cytogenetics we now require a knowledge of how one stage in the operation of the developmental mechanism takes its place in the entire series, dependent upon those which preceded it and conditioning those which follow. This analysis leads us back inevitably to the beginning stages in the organism and this in turn, just as inevitably, to the origin of the first phylogenetic unit. As a tool there remains for the latter only pure speculation.

Theories which outrun knowledge are properly suspect. They may, however, serve a good purpose so long as they are recognized for what they are, are clearly distinguished from facts, and serve to promote investigation which leads to new knowledge. Weismann's conception of chromosome functions was largely wrong, but it provoked a whole series of studies which greatly enlarged our knowledge of cytogenetics. At present the greatest hiatus in our understanding of biological processes lies in the field of development and differentiation. The results of combining genetical strains can be clearly foretold and the mechanism by which these are achieved is well understood, but as

to how the processes work we are entirely ignorant. Since there is general agreement that what is present in the individual is due to the experiences of its ancestors, we may reasonably expect to find within the continuous germ line from which individuals arise, a record of these experiences, otherwise they would not manifest themselves. There is hope, therefore, that comparative phylogenetic studies, combined with comparative ontogenetic studies, may serve to indicate how new characters arise and are perpetuated. The argument here advanced runs briefly as follows. Characters develop in organisms because of specific controls present in a continuous series of chromosomes; new characters manifesting themselves imply new controls added in the chromosomes; the peculiar actions of these controls show themselves ontogenetically by the sequence in which characters develop; this order reproduces, in general, that in which the characters appeared phylogenetically; the linear nature of chromosome structure represents the general order of new accessions to the chromosomes; in view of the fact that all Metazoa are fundamentally similar in structure and function, such differences as there are in the germinal mechanism must not be great.

B. Nature of Systems

1. *Systems Are the Varied Aspects of a Unified Cosmos*

An uncritical survey of the universe about us gives the impression of innumerable different kinds of objects. Very clearly, however, it became apparent that these naturally fall into different classes, and so there were conceived the Animal, Vegetable, and Mineral kingdoms. More careful consideration indicated that these groups are not coördinate, but that there is much in common between plants and animals. There thus arose the more reasonable classification of material objects into the two contrasting classes of organic and inorganic. The effort in this way to associate objects into classes because of relative degrees of resemblance in fundamental characters represents the basic step in the development of science. This analytical method, being extended in its application, led to the recognition of subdivisions in each of the primary groups. Organic things are obviously plants or animals; inorganic objects have properties which it is convenient to designate as chemical or physical. But as analysis proceeds we find that, after all, it is a fundamental unity which we contemplate

and that the groups which analysis reveals are largely subjective reactions to varied aspects of a common series of properties in all material things. While it is still convenient to call students of organic things botanists and zoölogists, it is often necessary to unite them under the title of biologists when they study properties common to all living things. Chemists and physicists still constitute separate groups, but their fields tend more and more to merge in the study of physical chemistry; and subjects such as biophysics and biochemistry indicate the links between the organic and inorganic. These considerations are all important in scientific thinking. They indicate definite limits to a field of study and determine the methods to be followed in further research.

The ultimate aim of science is an understanding of the essential unity of the universe; its method of procedure is to study systematically its varied aspects. This method naturally emphasizes diversities and suggests discontinuities that do not really exist. With increasing knowledge, the underlying unity gradually emerges and the apparently unrelated elements of the picture disclose their relations.

2. *They Form a Graded Series*

Dependent upon certain interrelations between cosmical elements, a series of systems manifests itself. There are no new departures, but each stage is marked by an advancing degree of complexity in the combination of elements from the preceding stage. The properties of the material combination are not to be inferred from those manifest at lower levels of integration, but inhere in the new type of relations set up. According to present conceptions, unity throughout the universe is due to the fact that all substances may be evolved from hydrogen, which in turn has its origin from electricity.

Ultimately, then, the universe is composed of charged electrical particles, variously combined and recombined at different stages, to form a series of systems. Within these there appear typical phenomena, each form characteristic of its state, but dependent upon those arising at lower levels. These facts indicate that in the Cosmos there is an underlying continuity which is interrupted at fixed levels to produce characteristic phenomena. Gradation is measured by the degree of complexity within the elements of the system and is inversely proportional to the degree of heat manifest.

3. *Systems Are Composed of Specific Units Which Are of a Serial Complexity*

It has been the experience of investigators in every scientific field that the process of analysis by which most of our knowledge has been gained, sooner or later carries us beyond the limits imposed by our senses. Visible structures are found divided into smaller and smaller parts and yet we do not find the ultimate units necessary to explain the phenomena observed. Inevitably there follows the assumption of still smaller units whose character and behavior we conceive to be of such a nature as to satisfy the requirements of the case. The tests for the accuracy of these assumptions are furnished by their conformity to resulting macroscopic phenomena and by the degree to which they permit prophecies of further phenomena. Practically they are not required to conform at once to all possible conditions, but are held valid if they are consistent within a limited range over a limited time.

A knowledge of the properties of the units of one system does not permit inferences regarding the properties of higher systems into which they enter as constituents. The characteristics of charged electrical particles alone would give no indications of the nature of the series of atoms which we know by the exhibition of their own activities. It would be impossible to conceive the molecular properties of sodium chloride even with full knowledge of the atomic nature of sodium and chlorine. In organic compounds often several having the same numbers and kinds of atoms possess very different characteristics, due to their different intramolecular arrangements.

The phenomena which physical units are required to explain reside within definite limits, e.g., there are a restricted number of chemical elements; they are related by increasing complexity in a periodic system, they combine in definite proportions, etc. Such aggregates form substances of definite, fixed and relatively unvarying properties, except for the most complex.

The basic elements are charged electrical particles—electrons, protons, etc., distributed throughout the universe. Only upon reducing the system to certain temperatures can these combine into atoms, each of which is supposed to be constituted of a nucleus, with a preponderance of protons, and a fixed number of electrons revolving about it in one or more orbits. The number and distribution of these elec-

trically charged particles determine the characteristics of the atom. The 90+ chemical elements have each a distinctive atom constructed out of the common building blocks of protons and electrons, but each differing in their number and arrangement. It is particularly to be noted that the units of one system constitute, by combination and arrangment, the units of the other. There has not been a fresh start with new elements in each case, but the higher system is constructed from the materials of the lower, i.e., the units of the lower system, by entering into combinations, produce others of a higher order which thereby gain new properties. Such combinations can occur only under certain conditions. Most of the universe exists at the electronic level and only in certain favorable spots can higher units be formed. Systems and units therefore are not independent or unique but are organically interrelated and their properties are relative. It is equally true, however, that the properties of any system are not to be explained directly by the nature of the units of other systems. These two properties—organic relationship and specificity—are inherent properties of systems. The former is due to the fact that they trace back to a common, all pervasive element or continuum—electricity—which must have within it potentially the properties of all systems. These potentialities are realized by successive integrations of units—electrons, atoms, molecules, radicals, each being constituted of the units of the next lower order. These units are distinguished by two characteristics—increasing numbers of constituent parts and increasing complexity of interrelation between the parts. The appearance of the higher units depends upon the existence of the antecedent lower ones —molecules cannot be formed without atoms, atoms without electrons. The complexity of functioning is proportionate to the complexity of the units and is definitely limited in each case.

C. Nature and Relations of the Living System

1. *These Systems Culminate in the Living System, Which Is Integrally Related to the Others and Dependent Upon Them*

In considering the relation of the living system to the others in the cosmic series the essential question is *"Does it have a correlative position of its own, or is it merely another expression of the forces operating at atomic and molecular levels?"* Since, as has been shown for non-living systems, their properties are due to the nature of their specific units, this question may be expressed more definitely in these

words: *"Are there peculiar living units upon which the properties of the living system directly depend?"*

Much of our difficulty in understanding the relations of living and non-living systems arises from our varied philosophical tendencies. Some persons seek for diversity, others crave unity. To one school this means that living things are something apart, whose actions are governed by vitalism or some other unknown force. For another, the phenomena of life are merely those of the non-living in a different form. Vitalism calls for some sort of special creation, the addition of something from without to make an insensate system operate; narrow materialism invokes chance, or accident, to shock reason into the inanimate. This antithesis usually represents the assumed possibilities for the creation of a living system. Rarely is it suggested that such a condition arises conformably and inevitably upon the underlying series of organizations having as units charged electric particles, atoms and molecules. The idea that reason and consciousness, the highest developments of the cosmic sweep, should develop by chance in a universe of law and order, does not at all accord with known facts.

A comparison of the characteristics of the various systems may help to answer this question of origin; but in setting out the apparent contrasts thus revealed, it is always remembered that we are not dealing with separate and unrelated entities but rather with the members of an integrated series of increasing complexity of organization. Within the more complex are found the units of the less complex unaltered in character, but existing in new relations.

Some of the suggestive comparisons may be given as follows:

1. Living systems are unstable, strongly reactive, functioning only under a limited range of physical conditions; non-living systems are relatively stable and slightly reactive under all physical conditions.
2. Living systems are slowly transformable in types, usually into ones more complex; non-living systems are relatively constant, but such changes as do appear are few and determinate.
3. Living systems preserve within themselves a record of racial experience by means of altered structure; non-living systems are relatively unaltered by time.
4. Living systems are strictly dependent for existence upon external conditions; non-living systems are relatively independent.
5. Living systems have the ability to alter other substances into

their own form for maintenance and growth; the non-living do not alter or grow in this way.

6. Living units can reproduce themselves; the non-living do not have this power.

7. Living systems are conscious of their surroundings; the non-living are not.

8. Living systems are cyclical in their expression; the non-living are fixed.

9. Living systems are embodied in discrete units, having a common but variable series of functions, but are unlimited in their combinations; the non-living have a basic set of physical properties, but each has a fixed and characteristic series of attributes.

10. New organisms pass through an extensive and phylogenetically reminiscent series of changes; non-living matter has no such marked developmental history.

11. Living systems are composed of many forms of non-living units; the non-living systems have relatively few units.

12. The chemical elements entering into the composition of the living system are common with those of the non-living.

13. These elements are individually strongly characteristic and consistent with the nature of the system which they compose, but the character of the system could not be predicted from a knowledge of the constituent elements.

14. The few and distinctive chemical elements composing living systems are common with those of the non-living; differences between them are therefore due to variations in the form of organization or relation of parts.

15. There are a series of non-living systems, each characterized by a distinctive unit—molecules are composed of atoms, atoms of electrons, electrons of electrical charges. For each step in the series there is therefore a unit to represent the characteristics of the system. The properties of living systems are superimposed upon those of their constituents; there must therefore be a living unit to correspond to the unique functions of the living state. Each advanced unit can be resolved into the lower units composing it, but the functions of the higher unit do not inhere in the constituent lower units. The properties of NaCl, for instance, could not be predicted from a complete knowledge of those of sodium and chlorine. Organic chemistry demon-

strates that the distinctive properties of a system are largely due to the form of organization of its constituent units.

16. Since lability and the capacity for rapid changes in non-living systems increase with the number and complexity of relations between their units, it must be assumed that living units have these properties to a marked degree.

17. The temporally continuous, nuclear portion of the living unit stores and propagates the experiences of the race; in the extra-nuclear part are manifested the results of racial experiences. These structural changes involve, at the first, the formation of various unit types—muscle, nerve, blood, etc. All grades of organisms come to have similar units, but they differ in the character of their development and in the complexity of the organization as a whole.

18. The method of exact duplication of units is manifest in the process of their reproduction by mitosis. The method of orderly and specific differentiation and coördination in development is complex and obscure. It involves, in large measure, questions of relation and interactions. Early units in a system may be interchanged and altered without visibly affecting their combined result. Later the units become unaltered, fixed in type. Time is a factor in development, as analysis demonstrates. If a factor does not operate when it should normally, the effect is altered or inhibited.

19. Differences appearing in the extra-nuclear part of a cell are due to forces set in operation in the nuclear elements. They are separable, mobile, definitely interrelated and specific in their effects. They must all operate in a definite order to accomplish normal results. To produce a given effect all the forces must operate, but in specific order and to different degrees and at varying rates. Organisms are, in the beginning, one-celled and their characteristic reactions are intracellular. When the organism consists of two cells a part of the reactions are intracellular and a part intercellular, the environmental reactions being largely unchanged. What the nuclear forces are able to accomplish depends upon the conditions of their operation, and, in development, these are constantly changing. In the very nature of the case, each change conditions all subsequent changes, according to an inherent pattern. The evidence shows that these controlling factors are intranuclear, and genetical analysis locates them in the chromosomes. It also demonstrates that they are in groups, within which

they have a linear arrangement. Space and time therefore inhere in the pattern.

From such comparisons it does not seem possible that there should be any break in the series of systems, neither does it seem at all reasonable that the unique properties of the highest system should be explainable in terms of the lower. Indeed all the known facts with regard to the relations between units and systems show clearly that the peculiar characteristics of a system are explainable only in terms of the units of that system, although these conceptual entities are but mental devices designed for that particular purpose. When, therefore, we are asked to depart from custom and to conceive the operation of a living system, not in terms of its own, but instead in those designed to explain phenomena of a different order, we are put in an entirely illogical position. If there is such a thing as a living system then it must, of necessity, have its own characteristic units to explain phenomena not evident at lower levels. At the same time it also takes its place conformably in the ascending series of systems and does not represent any break or departure from continuous development. It is not an accident or chance incident, but an inevitable development which external conditions make possible. This means merely that it is an integral part of the whole—the universe—not an isolated or extraneous creation.

What we know of vital processes has been gained largely from the study of cellular material, but there are recognized activities of a specific character which are due apparently to units of ultra-microscopic size. These so-called filterable viruses seem to reproduce themselves and by inference must be capable of metabolic action. They may represent a primitive stage in the succession of organic entities, or they may be a form of vital integration aside from the main line of succession. If they are vital, a complete explanation of such a form of organization would require their inclusion, even if they do differ from the great majority of forms. On the other hand this consideration would not dictate the type of explanation applicable to the higher developed and better known type, neither would it necessarily imply that such ultra-microscopic forms are the simple units out of which the more complicated are developed. So far as our observations go, vital processes are, prevailingly, directly and causally related to the organization found in the cellular unit. This may be functionally dissected and particular phases of the general activities assigned to

subdivisions of the cell, but the separate performance, by these subdivisions, of vital functions has not been noted.

Chemical and physical changes, in the cell, of a non-living character must not be regarded as causal in nature, but as only the accompaniments of the inherent forces of the biological unit. Since the cell is a material thing, ultimately composed of hydrogen, or electricity, there must occur in it all the forms of material activities determined by the presence of electrons, atoms, and molecules, but these do not determine the specific form of its manifestation, which is due to a higher type of organization. It is just as reasonable to attempt the explanation of phenomena due to electrons in terms of atoms or molecules as it is to try to account for living processes at the level of ordinary molecular action.

Living systems are accordingly definitely specific in organization, but almost infinitely variable in manifestation; they are constant over long geological periods, but never quite the same from time to time; higher forms are composed of units, each originally or potentially self sufficient, but eventually interdependent and coördinated; their units are constantly being worn out, in most tissues, but are maintained by the transformation of foreign substances into those of their own kind and are eventually replaced by the production of new units; these units, or cells, are, in turn, composed of lesser units having many of the properties of entire cells, including maintenance and reproduction. Reproduction of intracellular units precedes cell reproduction; no matter how diverse in size or complexity higher living systems may be, they are constituted of a like series of cellular units; in every case they typically pass through a specific pattern of development, commencing with a single cell; this cell is one of a continuous series going back to the origin of the type.

2. *The First Completely Functional Unit, in Most Organisms, Is the Cell, of Which There Are but a Few Types*

Through what stages biological units passed until a cell evolved, we do not know. There is little in the life history of the individual cell to suggest its phylogenetic background. It could not well be, however, that it came into existence completely formed. So complex a structure implies a developmental history which possibly may be suggested by studies of the lower plants. Logically it is to be expected that ultimate units first arose and that through subsequent develop-

ments they produced the infinitely more complex cell. From the simplest to the most complex the members of this developmental series were undoubtedly living things showing, among other properties, the inherent one of increasing the number of parts and specialization through greater and greater complexity of relations between parts. The first evidence of such tendencies would lie in spatial arrangements of similar elements in which both internal and external relations would be involved. Since primarily interchange of materials with the environment would be a necessity, some physical system permitting this must be present. In all later stages this takes the form of an interface or membrane and this was probably the earliest development in differentiation. Aggregations of units with extension of membrane surface would be the next step in complexity, leading to the formation of a vesicle at whose surface reactions would constantly occur. In this zone there would follow a stratification of substances, those near the membrane being most like the ones within it. Growth of the vesicle and continuation of reactions would build up an investment of materials which, in turn, would develop an external interface. In this way the formation of a nucleus and cystosome could be conceived. All stages in the process would be governed by the nature of the primary living units and not by chemical or physical conditions through which they work.

Within the nucleus lie a series of related centers of organization which have taken their present character from repeated reactions with a nearly constant environment. This distinctive character is not static, but rather a form of reaction. Alterations are due to differences in the rate and time with which a common series of functions are performed. If this is not true, the question immediately arises: *In what category do genic differences lie?* Do they result from varied chemical constitutions of molecular character? Are they of a physical type represented by variations in electrical charge or intensity? In answering such questions it must always be remembered that no matter how diverse the form of organisms they all perform but a few common functions. This raises directly the presumption that the causative elements in the system are similarly characterized. The fact that time is an integral factor in the organization of the system must be considered also. That this is true is shown in many ways, most convincingly perhaps by the fact that ontogenetic development follows the sequence of phylogenetic history. To reach any later stage

of complexity the same series of approaches has to be followed, beginning with a single cell. Here the situation differs markedly from that of non-living integrations of substance. A given piece of iron exists relatively independent of time and relations. Its properties are the same no matter where it is found in time or space. If it enters into combination with other elements it may be recovered in its original form without loss of character. So far as its essential nature is concerned it is self-sufficient. In these respects it is diametrically opposite to a living system which is entirely dependent upon its physical and chemical surroundings and which differs in its detailed form from instant to instant. Whether the ultimate units of the living system partake of its characteristics or whether they derive directly from lower systems is of the utmost importance in our thinking.

The cellular form of organization is not a necessity for life—it *is* required for the attainment of any high degree of development. It is the end result in the Protista and the beginning developmental stage in higher forms. Phylogenetic advance witnesses increasing degrees of complexity in the number of parts and their inter-relations. This progress is accomplished, not by developing more kinds of cells, but by increasing the perfection of their operation and by increasing the number and complexity of their inter-relations. Quite early in organic history, cells became differentiated into a few types each adapted to the superior performance of some one particular function. In every case action has to be adequate, so that functional performance does not differ greatly with phylogenetic advance except in the case of the nerve cells. These, in higher forms, become more perfect and diverse in their operation, although even here, it is not so much the performance of the individual nerve cell that counts as it is the perfection of the whole nervous system. Cell types do, however, show a wide range of diversity in form, but this is largely due to modifications in the cytosome. Taken as a whole every cell is essentially the same kind of unit. But, as a unit, it has none of the simplicity which appertains to the units of the physical sciences. Only by employing the term in a different sense can the cell be called a unit. It is so employed here only because it is the last organic subdivision which is functionally operative alone. It consists essentially of two parts—an inner, the nucleus, separated by a membrane from the outer, the cytosome, which in turn has its membrane or wall. The nucleus, in any cell, is much the same; the cytosome is little, or much, modified in accordance

with the peculiar function of the cell type. All parts of the cell are necessary for complete functioning, but the nucleus, although unspecialized in its activities, controls the activities of the cytosome, which do become specialized. In mitosis, the common form of cell reproduction, the entire process centers in the accurate, meristic division of the chromosomes within the nucleus. These bodies have been shown experimentally to carry the ultimate units which determine the form of body characters, which they must do by modifying the operation of all cellular processes, individually and collectively. Each cell, therefore, performs a common series of functions necessary for its individual existence and for the maintenance of the body wherein it lies, and at the same time exhibits peculiar properties, determined by the nature of its cytosome and called for by occasional requirements of the body. Cell activities are sometimes contrasted as individual and colonial but this distinction is not justified.

A cell, alone, performs a series of functions necessary for its existence; united with others into an organism it has a different situation to meet. Here certain ones of these functions or certain aspects of them result from the coördinated action of the groups. It is not from the sum of the individual cell activities, plus something contributed by the organism as a whole that the organismal functioning results—it is always that out of a necessary total of operations each cell contributes a coördinated portion. Its own welfare is served coincidentally in the total operation and not before or after it has served its colonial obligations. There can be no proper appreciation of how a multitude of cells work together as an organism if the members of the group are regarded as in any essential way independent. This is shown experimentally by separating early blastomeres. Kept together they perform each a part of the activities of an organism—separated, each becomes itself an entire organism. The functions of the unit and of the "colony" are therefore not different in kind but only in relation.

Organisms are machines adapted to perform a limited number of peculiar functions. Any organism, in order to exist, must be able to carry out these processes. In this respect, therefore, all organisms are alike. If organisms are thus similar, functionally, they must correspond structurally. This basic structural similarity is found to lie in the fact that all except the most primitive are made up of a common type of unit—the cell. On the other hand there is a tremendous di-

versity in the forms of the multicellular aggregates, or organisms. The great problem of biology is to discover how this great variability of aggregates can come about for the performance of a strictly limited series of processes, carried on by a common structural unit. It is obvious from a study of representatives of the manifold forms of organisms that there is a further order of structural resemblance—the different functions common to all organic types are, in each instance, performed by specific types of cells. Contractility is always the preeminent property of muscle cells; irritability resides in a special measure in nerve cells, etc. Diversity in organisms is due to differences in the number and arrangement of these various cell types. An explanation of variation must not therefore be sought in unique differences in structural units, but rather in the corresponding differences in the number and arrangement of a common series of cell types.

While these are the facts, it is equally true that each cell type is specific for the organism in which it is found—a muscle cell of an amphibian is genetically different from that of a mammal. Any other condition would require that cells be independent of their interrelations, whereas all of our observations show that they are entirely dependent on each other. When an ovum divides into two cells these become unlike in some measure; when a neural plate is cut off from the general ectoblast its cells are made thereby essentially different. In other words, the specific nature of any type of cell depends upon its particular relations, and these, in turn, upon its genetic history. It is therefore impossible to discover the nature of any cell or any aggregate of cells by studying it apart from its surroundings. This historical background reveals itself in the stages shown by a cell aggregate in reaching its final form, because in this process it follows the same path established by earlier aggregates of the same kind.

3. *The Cell, However, Is a Highly Complex Structure, of Uncertain Origin, Consisting Essentially of a Racial Portion* (nucleus) *and an Individual Part* (cytosome)

A cell, in many ways, is the most remarkable development of cosmic processes. A microscopic bit of living matter, a member of a continuous series reaching back millions of years in the past, it is at once a highly specific organization, and a comparable member of a common series found in practically all organisms. Part by part cells may be compared wherever found and these resemblances lie within visible

structures, which are so modified, according to a limited number of patterns, as to produce a common series of types in most organisms. Back of this demonstration of an underlying unity in all cells there lies, in each case, a high degree of specificity which makes the cells of each organism in some degree different from those of any other. Obviously this specificity is not due to the introduction of different elements, but rests in some modification of the properties and relations of elements held in common by all cells. As might be expected, in the presence of millions of different kinds of organisms, these specific modifications of cell parts rarely attain visibility. It is just here that cyto-taxonomic studies meet their greatest difficulty. What sort of modifications must be sought as indications of specificity in character? The cells of an organism fall into two classes: (*a*) those concerned with the immediate operations of that particular organism, the so-called somatic cells; and (*b*) those which stand apart from the cycle within the individual and look forward to future generations (the germ cells). Both are derived from the original fertilized ovum and have within them the same structures. With development, the somatic cells become progressively different, while the germ cells are relatively unaltered. In each case, however, the same specificity obtains. Functionally specificity consists in a particular manner of carrying out a series of processes common to all organisms. There necessarily exists structural modification to correspond with these functional peculiarities.

To characterize the cell as a "unit" is not to imply that it is of the relatively simple nature of units of lower systems. On the contrary, it is exceedingly complex. How did it become so? There are, unfortunately, few indications of its phylogeny. Was it a sudden creation, or was it evolved over long periods of time, becoming more and more like existing cells? In the formation of an organism we witness a succession of stages of increasing complexity which, in general, represent a series of fully formed organisms. We are thus provided with a criterion by which we may judge the succession of events involved in the becoming of any part of an organism, or even its whole composition. There is lacking such a criterion in a search for the origin of cells. Likewise, considering any organism, we can conceive something of the direction in which its type will evolve. On the contrary, cells are essentially the same, wherever found in multicellular organisms, and they show slight evidence of evolving into anything else.

A cell, plant or animal, is a cell, and betrays little of its origin or subsequent fate. In seeking an understanding of how cells came into existence we are therefore forced into pure speculation.

Because of their absolutely basic function in the constitution of organisms, it must be assumed that cells represent a crucial achievement in organic development. In the Protista, cell and organism are one. Up to that point, e.g., in the Bacteria, the organism has not reached the level of cellular organization, although it does have the two elements which constitute a cell, e.g., an inner portion of a chromatic nature and an external cytosome. These two parts seem to represent steps in development, for in cells the only directly continuous feature is the nucleus, formed by the chromosomes. Chromosomes are elements of individually specific character, which visibly reproduce themselves and so are organically continuous back to the beginning of their kind. This is further demonstrated by the fact that large taxonomic groups show, in the cells of their constituent individuals, exactly the same series of chromosomes. If thus, demonstrably, one portion of the cell can be conceived from the beginning, it logically follows that it is the original element in the system. Further it has been shown that this original element is precisely differentiated in the series of chromosomes and that these, in turn, are internally organized in the same precise, specific manner. When it is also found that these recognizable units stand in a direct causal relation to the developing characters of organisms, it cannot be doubted that here, within the nucleus, and directly in the chromosomes, lies the element of continuity in the system which leads inevitably back to its origin. For this reason it seems proper to designate the nucleus as "the racial part" of the cell, and since the nucleus is a product of the chromosomes, to consider that, within them, must be sought the original fundamental organic unit—bion, gemmite, determiner, gene, or whatever it may be.

Cells consist also of an outer portion whose activities are immediate in character and clearly related in structure to the function each has to perform. The contractile fibers in a muscle, for instance, are mechanically understandable as a means for producing motion. Cilia demonstrate, likewise, the correspondence between structure and function. All these cytosomic activities are evidently directly related to the welfare of the individual organism in which they occur. The nucleus, on the contrary, shows little essential structure change at

any time. Outside of certain obvious variations, characteristic of all cells, a nucleus is much the same wherever found. As Minot indicated in his theory of cytomorphosis, with age the cytosome becomes more and more predominant in somatic cells and the nucleus less important, even to complete suppression. In the germ cells, on the contrary, the cytosome is not directly concerned with the existing state of the cell, but is prepared for future events. It may therefore justly be said that the cytosome represents the individual and the nucleus the race. As will be indicated later this conclusion is suggested by certain relations of germ and somatic cellls.

D. Ultimate Units of the Living System

1. *The Racial Portion Is Demonstrably Composed of Ultimate Elements of Specific Action in Each Case* (genes)

a. Need for concept of genes
b. Possible origin of genes
c. Size and character
d. Position in the cell
e. Relations
   - Spatial
   - Temporal
   - Organic—alleles
     - homologues
f. Reactions
   - Intracellular
   - Extracellular
     - Heat
     - Radiation
g. genetic effects

a. Need for concept of genes. As has been shown, the material things about us belong to well defined system, each with characteristic attributes, and each with peculiar units whose relations and activities are used to explain the operations of its system. The question therefore at once arises: *"Does the living system differ from others and depend upon the units of other systems for an explanation of its functions, or does it also possess peculiar units by whose behavior its operations are conditioned?"* Logically there is only one answer to this question, for the essential functions of the living system are unique and call for correspondingly different units. But in non-living systems there is a direct and quantitative relation between unit and activity, so that exact predictions can be made of experimental results—is this true for the living system? It must at once be admitted that the extreme complexity of the living system makes any such

quantitative relations difficult of determination in most cases, and yet, in the behavior of contrasting characters in heredity, such a precise mechanism is called for. Since here is manifested the most characteristic function of living things, which results necessarily from the coördinated reproductive activities of its innumerable parts—cells, chromosomes, chromomeres, etc.—it must be that all the other activities which here find their summation, are likewise dependent upon determinative units, for upon them rests reproductive power. Indeed, in all the complexities and diversities of the living body, coördinated action, so necessary and characteristic, would be inconceivable without an underlying and pervasive unit type of organization. This is a logical requirement for the explanation of life processes, particularly in heredity, and has always been felt. All "particulate" theories of heredity recognize it and so we have the various assumed units of Darwin, Spencer, Weismann, and others. These, however, lacked association with experimental results and were not directly identified with known elements of the germ cells. It was therefore impossible to check the assumed operations of the units and this led Weismann to the entirely wrong belief that differentiation is due to a process of sorting out determiners, until only one kind remains in each fully differentiated cell. It is now easy to demonstrate morphologically that all cells are alike in the possession of a full complement of the nuclear elements which were thought to be partitioned out by progressive mitoses. Differentiation is due not merely to the presence of particular determiners, but to the positions they occupy in a series of reactions. Time is an intrinsic element in the processes—their nature is due to the operations and relations of distinctive units.

b. Possible origin of genes. Any attempt to conceive the origin of living units can be only pure speculation. They cannot be seen and what they are must be judged by what they do. This, however, is no unique situation but is true of the conceptual units of all systems—electrons, atoms, and molecules. The theories which postulate such entities are of the greatest possible help in advancing our knowledge, and whether they are accurate in detail is of minor importance so long as they serve this purpose. We seek eventually full knowledge, and our only hope of gaining this is through the formulation of hypotheses which aid our thinking. Therefore, although we well know that there is little to guide us we must try to imagine how living units arose.

The first point to be noted seems an assured fact in that they contain no unique materials—if all the cosmos can be resolved into electricity, so can they. Next it must be true that they are very small, for the course of all developmental changes is from simple to complex, from few parts to many, from small to large. Again, since they must possess the attributes of the system which they are designed to explain, they must have come into existence in reaction to certain, limited and necessary conditions. Only when the earth presented these conditions could they have formed and, so far as we know, they cannot exist anywhere else unless these are realized. Living units must be such as are in a constant state of reaction to surrounding conditions—these are two aspects of a common problem and must be considered together. Relations, then, as well as inner constitution are significant —indeed it is probable that the peculiar nature of a living unit is due to some unique reaction between an unstable, large molecule and the physical conditions under which life exists.

If we attempt to conceive what may be these unusual circumstances, we must recognize that they arose conformably out of preceding conditions and are in no sense accidental or strange. While, however, non-living units may exist with much fixity of form and relative independence, living units are in a constant state of flux and necessary reaction with external elements. Since for maintenance, this reaction is necessary it must be that it formed a requirement for the origin of such units. Since all we know of living things teaches us that they survive in proportion to the measure of their adaptation to external conditions, it is no doubt true that final attainment of an effective organization was preceded by many unavailing stages. Basically, of course, this new organization was one which could utilize solar energy to transform foreign substances over into its own likeness. A unit, however small, would necessarily present one aspect to this source of energy and conversely possess an opposite aspect, lacking such a direct relation. Inherently, therefore, a differential relation must exist, between the unit and its environment, such as is observable in larger units, e.g., an egg floating in water. There results a corresponding difference in function, and since constant interactivity with the environment is the distinguishing characteristic of the living unit, this polarity is both inherent and highly significant.

Assuming that the origin of an organic system is a single unit, a

point in space, how is it possible to conceive the consequent formation of so complicated a structure as a cell? Usually in following out the history of any part or element in an organism we can find a phylogenetic series, running from simple to complex, wherein it is possible to trace the successive historical stages. This sequence, in turn, is mirrored in the ontogeny of the individual. There appears to be no such comparative picture available to aid us in conceiving the origin of a cell. Certainly in any Metazoon nothing but perfect cells could be anticipated. It is more than probable also that primitive cells were capable of direct conversion of inorganic materials into organic. Therefore, it may be assumed that approaching developmental stages would proceed along a similar course. Logically this would lead back to the conception of the original unit as a molecular aggregate capable of utilizing solar energy to transform other substances into its own likeness. Metabolism would necessarily be followed by growth and reproduction in any living thing.

Still, in all this, there is nothing to suggest how a coördinated system of differentiated parts would follow. Since, however, it is an inherent property of living things to change, and, prevailingly in the direction of greater complexity, with resulting increased precision of functioning, these simple units must have eventually become more complex. Why this is so is no more apparent than why metabolism and reproduction should characterize living things, or why corpuscles should unite into atoms or atoms into molecules. It is inherent in a form of reorganization that certain properties should necessarily represent it externally—once such a physical configuration is reached the properties inevitably accompany it. To say, therefore, that an original living unit must show the properties that characterize all living things is merely to define such a unit. Moreover, it is obvious that changes which have survival value are those which increase the precision of reaction between a unit and the conditions under which it exists. By and large, increased complexity and precision of action make for survival. This can be no chance condition. In some way or other it is inherent in the nature of a living system, and it merely means that instead of varying equally in every direction during successive embodiments, it varies prevailingly in one. The existence of polarity in a system would necessarily be followed by such a unidirectional change. Living things are well characterized by polarities

and symmetries and these properties appear in their parts down to the limits of our observational powers. The chromosomes within which the genes lie are definitely polarized and in such manner as to suggest strongly the temporal relations of their parts.

Direct physical continuity would seem to be a necessary quality of the element within a system which is determinative of its character. Such an element would go back to the beginning of the system, necessarily. The only parts of cells which are observably continuous from generation to generation through their individual reproduction are the chromosomes. Not only as entire bodies are they thus continuous, but their constituent parts, the chromomeres, are also continuous through individual reproduction. Within these chromomeres the genes evidently lie and presumably continue the process of particulate reproduction. A gene existing today would therefore be the direct material descendant of the first one of the series. As an individual dies all cellular parts disappear—the only continuous members are the chromosomes of the germ cells. Demonstrably their continuity back to the origin of the system inheres in the chromosomes. But there is here postulated the existence of the system—the cell; what of its parts? May we assume that the ultimate living units, now identifiable with genes, existed before cells came into being? No observations are at hand to aid us in answering this question; we must proceed again on pure speculation, if we would gain any knowledge of the existence, character, or history of antecedent cellular elements. Whether such an effort be at first successful is not of prime importance if it leads to constructive thinking on the problem.

Let us then assume that the first step in organic development was the formation of a primary unit. This, as we have noted, would be a molecular aggregate of ultramicroscopic size. We should conceive that it originated inevitably from conditions within the constituent molecules in response to others external to them. This dual set of circumstances seems to be a necessary assumption, from all that we know of living systems and their essential reactive nature. What these required conditions were we have no way yet of knowing any more than we can understand why a particular constellation of electronic corpuscles were required to produce a certain chemical element. While ultimately necessary for complete understanding such knowledge may meanwhile not be indispensable for conceiving subsequent steps in the

history of the assumed units. By definition such a unit would be one in constant reaction with surrounding elements. About it would be a field of activity differing from that surrounding non-living units. Because of the reactive nature of the unit, there would be, from the first, a polarity manifest in this field which would coincide with an axis of the earth at that point. Distally, direct exposure to solar radiations would result in a higher energy expression than proximally, accompanied probably by an electrical polarity.

Such conditions we find, e.g., in an egg floating in water, therefore an assumption of differential polarity is consonant with what we know of larger units. Reproduction, being an inherent property, would result in the production of more units. These might either separate or form aggregates. Because of the polarity distinguishing the unit it is conceivable that the plane of division might determine separation or union. If, e.g., the unit should divide along a plane passing axially there might result equivalent halves. If there should exist a bilateral symmetry, which would be a reasonable assumption from what we know of larger units, then this would almost certainly be the case. Consequently we might suppose that this would be the common form of division, representing, as it would, a mode of inner order.

On the contrary, let it be supposed that a unit might occasionally be subjected to such unusual conditions that it would be obliged to divide transversely, thereby separating proximal and distal regions. The resulting two elements would then be unlike, although repeating in each something of the original polarity, proximal and distal. If there were forces present, such as distinguish electrical polarity, then the two units would tend to remain united, and one, in relation to the substratum, would be proximal and the other distal—the beginning of a chain would have been made—such a chain as we find developed in the linear organization of chromosomes. In the cleavages of eggs both equivalent and differential divisions occur, so that their assumption in lower units does no violence to our experience.

It is true, however, that in the division of chromosome parts, which we are here trying to correlate with hypothetical units, we see only equivalent division—there is nothing to correspond with a segregation division except the remotely similar one in meiosis. And yet we know that in the evolutionary series new somatic elements arise and, if our theory of genic control is correct, there must be a corresponding

production of new genes. True these might come from combinations instead of by divisions, but that is not what we see at other organic levels. To the objection that segregation divisions have not been seen there is the obvious answer that they are rare occurrences and even if they happened in a complex system they might not be noted. There is the possibility that a comparative study of the chromosomes in some group might reveal the gross effects of such a type of division, for certainly, after the establishment of a cellular system, combinations of ultimate units could not occur. This, of course, is on the assumption that such ultimate units lie in the chromosomes and are, in some way, identifiable with genes. Such an effort to fathom the nature of chromosome integration is, in fact, a main feature of the series of comparative studies being made upon the germ cells of the Acrididae.

If it be granted, then, that by a process of occasional segregation division a gene string, identifiable later with a chromosome, arose, we are still a long way from comprehending the origin of a cell. There first arises the question of how so complex a system, including many chromosomes, could come from a single element. In all the behavior of cells there is here little to aid us. True, in rapidly dividing cells each chromosome forms its own vesicle and there is no common nucleus. It is also observable that often the nucleus is the result of a coalescence of separate chromosome vesicles. Then there is the case of Llaveia reported by Hughes-Schrader where there is no common nucleus at any time, or even a spindle at mitosis, but where each chromosome is a separate entity. We have also the knowledge gained from the intensive study of Drosophila that chromosomes do not differ, in their included controls, by regions of the body affected, or by any other recognizable segregations of function. There are, e.g., genes affecting the eyes in each chromosome and similar diffusion of function is found for other characters. It seems clear that, although there are unit relations between genes and characters, the whole system is intimately involved in the expression of every function.

With respect to the origin of chromosomes this might mean that a single chromosome originally sufficed to condition the development of a body, but that as more complex responses were required, other chromosomes arose. A serious objection to this view is that there is no relation between taxonomic standing and chromosome number. Within the Protozoa there is, e.g., a wide range of chromosome num-

bers. It is to be remembered, however, that in stages preceding actual cell formation conditions might have been quite different. There can be no doubt that the formation of even the simplest cell represents the summation of exceedingly long and involved processes. If, therefore, we disregard for the moment the question of chromosome numbers, we can possibly see how a chromosome originated and how it produced a vesicle by taking up water. Reactions between this vesicle and varying external conditions would cause greater condensation of substances on their surfaces, where necessarily they would come on their way into the vesicle. In time an order, controlled by the living elements within the vesicle, would result and a cytosome would be formed. There is expressed by these assumptions the view of control units being gradually removed from contact with the environment and operating indirectly—first through a vesicle membrane and then through a cytosome or even a cytotheca. This is what we see in a functioning cell—did the condition in fact arise as the summation of progressive changes, in some such way as that suggested, or was the first cell at once a completed product? All our experience would eliminate the second possibility and support the view of developmental processes. Essential to our view of cell action is the idea of a complex of elements functioning coördinately under the control of various inner elements whose character has been determined through repeated experiences of a similar character.

c. Size and character of genes. Genes are assumed to be discrete material particles, of ultra-microscopic size. Therefore, whatever knowledge we have of them must be inferential. Several estimates of their size and number have been made, and one of these is that a gene is about one-quintillionth of a c.c. in size or is equal to 15 protein molecules in volume. The number present in the germ cells of Drosophila has been estimated to be not less than 14,380, and some investigators have calculated an even higher figure.

The number of genes is reached by observing the incidence of mutations under the action of X-rays; the size by dividing the total amount of chromatin by the number of genes. Even though exact numbers and dimensions may be uncertain it is clear that genes approach the molecular in size and that their numbers are within conceivable limits. Restricted as this knowledge is, it nevertheless is important in defining the field of our speculations regarding genes.

Very important is it to know whether they are molecules or not—if molecular then their activities would be governed by chemical laws; if supramolecular, then their functions fall within another realm. Since these functions are known to be of another order than those found in non-living systems, it is not surprising to find the units upon which they depend of a new degree of magnitude. It is also known that genes do not exist as separate elements, but that they are elements in a fixed and coördinated system. These are all important points in seeking an understanding of the nature and origin of genes. Not only the intrinsic nature of the element itself has to be considered, but also its position in the system. This naturally gives rise to the question *"Are all genes alike and are their differential effects due to variations of relations within a reaction system?"* To a very large degree they must be alike, for they can possess no functions not common to all living things, but at the same time these might vary in time, place, rate or degree of their action in the system. The so-called "position effect" would be an instance of such a type of variable effect. Also it is known that the results produced by the same gene at different stages of development in the individual are not the same. Furthermore to produce any one effect all the genes in the system must coöperate. From these considerations it is obvious that the extreme simplicity which characterizes all early explanations of factors in development and which sees a direct relation between factor and character, cannot hold. Genes produce their effects as elements in an extremely complex reaction system and any effort to comprehend their nature must have due regard for this primary condition. Most genetical analyses deal with the final effects of the operation of the system and associate each gene with some finished character, thus suggesting a simple relationship between the two. Enough is now known about gene action to make it possible to state with assurance that for the production of any one character all the genes are necessary and, conversely, every gene has multiple effects in development. It is only a statement of a fact to add that *time* is an inherent factor in this problem.

d. Position in the cell. With regard to genes, here, at least, is one topic upon which a definite statement can be made. All modern theories of heredity are based upon the known behavior of the chromosomes, whose movements in maturation and fertilization correspond precisely with paternal and maternal character inheritance. The basic

phenomenon here was first reported by Pfitzner ('82) and commented upon in its theoretical relations. He observed that each chromomere accurately reproduces itself in mitosis so that each chromosome is thus precisely duplicated. Roux seized upon the fact as evidence that structures, so exactly duplicated, must be of primary importance in cell economy. Immediately Weismann, adopting this view, located his idioplasm in the chromatin and elaborated his theory of the germ plasm which had so profound an effect upon studies in heredity. This general linear organization of chromosomes was found to be universal, but Wenrich ('17) was the first to demonstrate that it is precise and constant through the species for any particular one. This cytological condition has been found reflected in the genetical operations of chromosomes by many detailed and painstaking analyses during the years of the present century. Chromosome maps have thus been made possible, which can be checked against actual structures. Correspondences thus revealed are very exact, so that now the location of the genes in the cellular system is definitely known. Recognition of this linear order, however, has not, up to the present, received any theoretical explanation beyond the evidently practical one that it makes exact division easy.

d. Relations of genes. These may be considered under the rubrics spatial, temporal, and organic. For each chromosome the contained elements have a linear order proceeding from the proximal end, where is located the acromite, to the distal. This is no chance arrangement, but is fixed and definite. Genetical analysis shows this constancy for the assumed genes and cytological observations demonstrate a corresponding structural definiteness. This linear order and precision is the outstanding feature of chromosome organization and must connote a function of corresponding significance. The order within the chromosomes is matched by a corresponding definiteness of relation between members of the complex. For the individual, the species, often the genus, and even the subfamily, the number is the same and there is a seriation which, as yet, is imperfectly understood. There is also a definite observable relation, during at least some portions of a cell's life history, between the chromosome and the cytosome. Such an organic integration must necessarily always exist, but the polarization of the auxocyte chromosomes and the accumulation of a cytoplasmic secretion opposite their ends is a specific indication of this. Doubt-

less there are many interactions between the elements of so complex and highly attuned a system as that found in the cell which will be revealed only after much more subtle analyses. What is now clearly evident, however, is the existence of infinite possibilities of such relations. Spatially the genes are found to be members or parts of a fixed number of groups located within the nucleus and operating specifically to control cell activities. In some way it is not the mere presence of these genes that is responsible for their functioning, but, beyond this, there is an order which is primarily significant.

e. Relations. When the operations of living things are studied the element of sequence, or time, at once becomes manifest. There is nothing more obvious in the economy of life than the element of repetition in sequences, ontogenetic and phylogenetic. A given stage of individual development is reached only by passing through a fixed series of preceding stages which correspond, in general, to stages which the group has known in its evolution. Complexity is reached only by an approach through an ascending series of increasing number of parts and relations. A record of multiple like experiences is made in the continuous germ plasm which unrolls in the same sequence during individual development. Time is thus no abstraction in organisms but a real element of their being. It cannot be abbreviated or eliminated but must duly manifest itself each time an organic unit emerges. Indeed, life is evident only as a succession—a series of waves, increasing in height as they progress. The inorganic components are lifted into the new level as the wave comes and drop back into the old one as it passes. Life is essentially reaction and time is its measure. It finds a spatial record in the germ plasm where time and space are potentially one.

f. Reactions of genes. Unfortunately, little is known about this subject, so far as method is concerned. The only possible way in which genes can produce an effect is through their operation in the system, of which they form a part. This system is of the utmost complexity, but clearly it follows in its activities an invariable order—a late step is possible only after a series of predetermined earlier ones is completed. In a system of time-space points certain configurations are required before others are possible. If any of these are experimentally deformed the results are altered. In the activities of the genes lies the ultimate secret of life, so that there is little hope now

of learning much of their behavior. Still the possibilities of their action must be canvassed and experiments devised to test them against each other.

Indeed some of the earlier conceptions have already been disposed of. The most outstanding of these was that advocated by Weismann, who conceived each determiner to act through a direct and sole relation with one character or type of cell. Instead of increasing simplicity of relations, thus postulated, there is instead, all things considered, increasing complexity. For the individual cell it is true that, as differentiation proceeds, limitation of reaction becomes greater, until finally it settles into a characteristic type. There results then the great problem of how coördination is established between the multitude of diverse cells in an organism. Obviously, it cannot be of the direct form possible within a single cell where the genes are in intimate relation with the rest of the cell. And yet the fact that all the cells carry the same gene complex indicates strongly that in some profound way their coördination is necessary for that of the whole organism. This is practically demonstrated by the fact that if a single gene is lacking in a gamete it cannot produce a normal individual. In some way or other the coördinated action of a similar mechanism in each unit is required for organismal unity.

There are, of course, the coördinating mechanisms of the nervous and humoral systems, but these had first to be developed and even before their existence in the body, its activities proceeded harmoniously. Since all the genes are direct descendants of the original set in the zygote and are the same in each cell, there is manifest a tissue of controlling forces having each the same character, method of action, and position in the series. Conceivably action in any one active cell would be reflected in some measure in all the other cells in the system. What the nature of this action may be we do not know, but certainly it is of a nicety and complexity far beyond that of any other known system, for consciousness emerges from it—and consciousness looks back upon itself and seeks understanding. Perhaps what we call consciousness is the summation and integration of all the intra-cellular forces of coördination organized in the most responsive of cells—the nerve cells. It is much more in accordance with the peculiar conditions of the living system to think so than it is to attempt an explanation of consciousness and coördination in terms of physical systems.

But the reaction of genes within the living system is impossible apart from the environment which conditions their existence. Life, in essence, is reaction. Evolution is a summation of reactions recorded in the genes and repeated endlessly in the sequence of individuals constituting a race, where, in time, new elements arise to make more perfect the expressions of these reactions. There has been much discussion as to whether or not the operations of these environmental forces are direct upon the germ cell mechanism or indirect through the body in which they are, for the time being, housed. There seems to be no reason to exclude either method. Experimentally it has been found possible by radiations to increase greatly the rate of mutations normally manifesting themselves. It is inconceivable that the complement of genes present in the germ cells could remain unaffected by changes in the similar groups within the body cells. At the same time the germ cells are relatively isolated within the body and their reactions would be of a different order. Nothing is more clear with regard to organisms than that they and their parts are understandable only in terms of purpose—of a means toward an end in each case. That end is always achieved through the utilization of circumstances external to the organism.

As the environment changes, so does the organic type—prevailingly in one direction—from simple to complex, from a low degree of consciousness to a higher; from contact with and use of immediate environment to a wider and more cosmic relation; structurally from a small and diffuse nervous system to a large centralized one. Curiously, as it now seems, the genic or nuclear structures do not observably differ as complexity of structure advances, but, in the cell, the cytosome becomes more involved and differentiated. But even here we do not have a visible genic measure of advancement. What, above all, marks a high degree of phylogenetic development, is the number and extent of interactions between the constituent elements of the nervous system. Doubtless there are corresponding structural differences in the germ cells, but these are not so obvious. Doubtless, also, the nuclear structures upon which cell activities and relations depend are altered, but not recognizably. Potentialities inhere within the genes—these are realized in the structures and activities which they condition.

Of what nature are these potentialities—may they be looked for in

new types of genic aggregates? Obviously not, for the germ cell mechanism which represents always the simplest stage of organisms, shows no unusual developments even in man. All developments seem then to arise from a common type of control mechanism, but we know that if our theory of causal relationships between controls and characters is correct, genes must show antecedent changes when new results are achieved. If these are not visible in new or unique forms of elements in the causal series then they must consist of additions of similar type which thereby modify the operations of the system through increased complexity of processes. This, indeed, corresponds to what we note in organisms themselves. All are alike in the functions they perform; all are alike in being constructed of cells which are modified into a series of types for the performance of these common functions. The changes which we do see consist in the additions of more parts which have accompanying differences in details of structure, and in increased range and complexity of interrelations. If we are in search of an explanation of how the germ cell mechanism operates, it is of the first importance to realize this, because we find only what we are looking for. We must not, therefore, seek for new cells structures, but rather for modifications in the form and integration of a series common to all. The range of differences is not great and mainly in the way the genic material is aggregated into chromosomes and the rate at which changes in extension and concentration of chromatic material occurs.

g. Genetic effects of genes. By accurately controlled breeding experiments, particularly upon Drosophila, precise relations have been demonstrated between the behavior of the nuclear materials in the germ cells and the characters of parents and offspring. In particular it has been found that the number of characters transmitted in groups and the number of chromosome pairs are identical. Moreover, the relative sizes of the character groups and of the chromosomes correspond. It is apparent also that the genes are arranged in a linear series, which, in each case, can be correlated with a particular chromosome. No more precise a coördination between structure and function has been demonstrated in biology than that of the germ cells. And yet this demonstration concerns almost exclusively the end results of the operations involved. Parents exhibiting contrasting adult characters are bred together and their offspring are listed for the presence

of similar characters. There is nothing to indicate the method by which these ends are reached—nothing to show how the mechanism works. It is apparent enough, however, that any one character is dependent upon the operation of all the genes and therefore that each gene has manifold effects. If this is true for end results, where it has been demonstrated, then it must be true for all stages of development leading up to the finished product.

There yet remains for solution the central problem of biology—the method by which one cell gives rise to a multitude of other cells, following a fixed pattern, to eventuate finally in a specific type of organism. Since we cannot actually observe the behavior of genes during the development of an individual, how can we hope to learn their relations to developing characters? There are two ways that suggest themselves (*a*) modify the course of development experimentally and (*b*) observe the variations normally present within a group of related organisms and in each case seek evidences of differences in the germ cell mechanism. Nature has performed an experiment which, if understood, would carry us far toward a comprehension of this problem. In all higher forms the individuals of a species occur in two forms—males and females, i.e., a common group of characters is presented in two aspects. Accompanying these somatic differences are clearly observable corresponding divergences in the genic mechanism. If we could trace the progressive and parallel changes in soma and cell elements, we would have the basis for an analysis of development in relation to genes.

2. *The Arrangement of These Units Is Linear, Which Represents a Sequential Inclusion in the System*

The most striking feature of chromosome structure is the linear arrangement of the constituent chromomeres. It was this fact and the observation of Pfitzner that each granule in mitosis is accurately divided which led Roux to the conclusion that these conditions fulfill the theoretical requirement of a germ plasm. When later genetical analyses demonstrated that assumed causal factors correspond in their order and behavior to the observed conditions in chromosomes, the identification of the site of genetical determiners in the chromosomes became highly probable. Finally, when comparison of the genetically derived linkage group maps and the cytologically observed giant chro-

mosomes of Drosophila exhibited almost identical numbers and relations of parts, the demonstration of the presence of the genetically potential elements in the chromosomes was complete.

What, then, is the meaning of the linear order of parts thus revealed? First, it must be noted that there is a profound significance in the fact that the determinative units are formed into groups and do not exist independently. Conceivably they might do this, but the possibility is never realized. One result of this condition obviously is the formation of character groups, which manifest themselves genetically and correspond in number and size to the varieties of chromosomes present. Linkage thus receives an immediate explanation and what is present in the individual finds revelation. But how did this order come into being? It has long been noted that in a general way the developing stages in the individual recapitulate the phylogenetic history of its group. If characters result from the differential operation of specific factors in the germinal mechanism, then their effects succeed each other in time. With our present knowledge of the complexity of developmental processes, it is impossible to accept earlier, over-simple conceptions of a single and direct relation between factor and character. What we observe cytologically is not a sorting out of chromosomes during development, but a constant and accurate preservation of the complex found in the original one-celled zygote. We must therefore believe that all genetic factors are perpetuated.

At the same time it is just as obvious that what happens at one stage of development is different from what occurs at other stages. The conclusion is inescapable, therefore, that any one action is dependent upon preceding actions. But if actions are due to material elements in succession, this directly argues for a temporal addition of these elements to the system and the nature of this seriation is determined by the phylogenetic relation of corresponding characters. From this it follows that the linear order of determinative elements in the chromosomes represents a temporal sequence in their accession. If this argument be granted, is there any evidence to suggest how this order runs? In the absence of any adequate analysis of character relations in developing organisms there is as yet no guide to the solution of this problem.

The sequence in the structural order of these elements must therefore remain a matter of inference. But while it is true that no in-

flexible order is to be expected in so labile an organization, there are reasons for believing that, in general, the order of accession of new structural elements in the chromosomes is from proximal to distal. Suggestive here are the following: (*a*) The acromite is a permanent and necessary part of the chromosomes—its loss makes participation of the chromosomes in mitosis impossible. No matter how small the chromosome it must have an acromite. It may therefore be presumed that this proximal portion of the chromosome has been there from the beginning and that additional elements are added distally. (*b*) This portion of the chromosomes is, in meiotic prophase, most intimately associated with the cytosome, at which point specific secretions are often found. (*c*) The acromites, at this time, are all grouped together on one side of the nucleus. (*d*) In movements of chromosome elements the proximal portions of the chromosomes take precedence. (*e*) Associations of chromosomes occur principally at their proximal ends. These are of various sorts. In some cases the "proximal granules" fuse into a complete chromoplast. The chromosomes of Drosophila are proximally imbedded in a comparable structure and here a similar set of bands runs through all the chromosomes. (In this portion of the chromosomes few genes have been located and it is sometimes said to be genetically empty.) Two things might be true regarding the proximal region of chromosomes: (*a*) If it be the oldest part it would be least differentiated and all the chromosomes would be similar to a larger degree than elsewhere and (*b*) it would be concerned with the development in the early stages, or with somatogenesis, and little at all with the superficial characters used in much genetical work.

If new characters mean new factors and these are added from proximal to distal, does it mean that only the terminal members are young? That would not follow necessarily. If additions are made by a qualitative cross division, any particle anywhere in the series might theoretically be involved, but since, by assumption, the proximal ones are concerned with the more general and primitive features of organization, changes here would be rare and concerned only with phylogenetically older characters. Thus, while a rigid order of succession is not to be anticipated, in general new additions to the factorial representatives would prevailingly be distal.

There are known facts, moreover, which speak for internal changes

in chromosome structure. Parts of different chromosomes are interchanged and come to lie in altered relations; within one chromosome a segment may be inverted and thus reverse the order of its elements; a fragment from one chromosome may be attached to quite another and so disturb the normal order. There is some evidence to indicate that such disturbances may be followed by genetical changes. It is also a question as to how long such evident alterations in so long established an order may persist, for it must be assumed that it *is* well established because the cytological picture revealed in any organic group presents remarkable constancy. There is nothing there to indicate that marked changes can survive. Another complication enters into the picture also. Instead of a single linear series of determiners in a cell there usually are many, and accessions may be made to any of them. That they did not grow equally is evident from the difference in size of their material aggregates. Since, as is well known, the effect of any determiner is merely that of a differentiator in a coördinated system, it is difficult to conceive how it might be called into being. As yet no reason for the existence of more than one chromosome has been revealed by genetical analysis. There is no segregation of influnce in the chromosomes according to any recognizable morphological plan. The accessory chromosome has a determinative effect, in coördinated action with the others, upon sex-characters, but, since these represent merely alternative expressions of all the characters of the body there is not much to suggest how the action occurs—and this is true of every case of differentiation studied. Sequence of effect would not necessarily lie between elements within the same chromosome, and a factor, wherever placed, might come into specific action at its normal time. Because there is a precise organization of all cell parts, it hardly seems possible that any disturbance of relations could occur without some effect, although this might not evidence itself in the expected manner. In development a sequence of characters is clearly apparent, proceeding from general to particular, and this similarly is the order phylogenetically. It seems, therefore, impossible to escape the conclusion that factors manifest their particular effects in the order of their inclusion in the system.

3. *The Nature of Their Action Is Not Single, Restricted, or Unique, but Continuous, General, and Temporarily Distinctive*

In all particulate theories of heredity and development, there is

evident a tendency toward over-simplification. A given factor, at one time and in one place, is conceived to be responsible for a single specific effect. This was strikingly shown in Weismann's analysis of the problem, as well as in Darwin's and Spencer's. Later, Mendel's conception of unit characters seemed at first to point to such a relatively simple condition in organisms. But, unfortunately, the further our analysis proceeds, the more complex, it is evident, is the system we study. Already it is demonstrated that the idea of one factor and one result is impossible. Also it is clear that the organism is not an assemblage of separable characters, some individual, others specific, generic or family, in value. What we roughly call characters are features of structures of every degree of complexity, constantly altering during development and finally reaching a balanced state in maturity.

In seeking an explanation of the relation of factors to characters we must realize this constantly altering nature of the system. The ultimate units were themselves originally at once factors and characters. United to form larger units—chromosomes, cells—these in turn became characters. Cell layers, tissues, organs and systems became successively more complex characters. Always there came about more elements and a greater range of interaction between them, but always the elements have the few and characteristic properties of living things. An element acting alone, or performing the same function in a group of many others, would necessarily produce different results in the two cases. It does not require the conception of a multitude of unique factors to explain the many forms of organisms. A relatively few types of factors reacting together at different times, places, and rates, under constantly altering conditions would suffice to explain the varied results observed, particularly if it is remembered that organisms perform, at most, only a few common functions. From the nature of the case we must conclude that: (*a*) a factor is necessarily always operative; (*b*) its effect influences, in some variable degree, the operation of the entire system of which it is a part; (*c*) it has at some relative period in development, a more marked and characteristic effect than at other times; (*d*) individually it is dependent upon the functioning of all the other elements in the system; (*e*) the end result of the operation of the system is not the sum of the effects of all the parts, but a progressively attained state of complexity wherein

a multitude of parts interact coöperatively with each other so that the incomprehensibly large number of separate actions seem to be the operation of but one unit.

These facts are necessary for an understanding of the manner in which both the individual units and the whole system operate. A tendency on the part of some investigators to focus their attention upon the activities of single elements while others view them collectively leads to antagonistic schools of thought for which there is no excuse. It is equally certain that there are separate parts in organisms with distinctive functions, and that conversely it is possible for these to operate only in relation to all other elements in the complex. Appeals to such conceptions as "the organism as a whole," "the cell as a whole," and, finally, "the chromosome as a whole" based upon the obvious truth that each "is more than the sum of its parts" are disingenuous. Of course they are—as every biologist knows—the coordinated and progressive summation of the activities of their parts—not the arithmetical sum of them, as is implied in the statement of the question. Each part has a characteristic action which it can manifest only in coöperation with all the other parts and distinctively only at a particular time. Progressively there are built up more and more complex situations wherein the same series of determinative factors operates, producing successively different results because of the particular circumstances under which it successively works. Time is an essential element here. The individual sequence is invariable and this derives from phylogenetic experiences. Added characters conceivably result from new factors introduced into the system or form altered relations of those already existent. In view of the fact that there is nearly always a genic basis for each character it is probable that most new ones are due to added genes.

E. Organic Combinations of Units

1. *The Ultimate Units Are Organized into Definite Larger Units, the Chromosomes, Which Are Self Perpetuating, Phylogenetically Constant, and Ontogenetically Effective in Relation to Body Character Groups*

Ultimate units do not exist free in the cell, but form, or are contained in, larger nuclear units—the chromosomes. On a priori grounds there seems to be no reason why genes should not function as separate

entities. Indeed it seems reasonable to suppose that they may do so under primitive conditions. That they occur, in all higher cells, as parts of definitely organized chromosomes, doubtless represents a functional development. Experimentally it has been demonstrated that the control groups correspond in number to the pairs of chromosomes and further that the number of characters in a group is roughly reflected in the size of the corresponding chromosomes. By observation it is possible to determine that chromosomes are constant in their inner structure and are also individually different. In sexual reproduction, the behavior of characters, individually and collectively, corresponds to that of chromosomes and their elements during meiosis and syngamy.

It is thus apparent that the relation between the structures of a body and the racial record of the germ cells is a material one. Additional elements of structure in the soma therefore mean, generally, corresponding new controls within the chromosomes. Is there anything in the structure of the chromosomes to indicate how these additions are made? There are two possible ways in which such additions could be made to chromosomes—either by externally formed structures, e.g., chromomeres, being added to a chromosome or by the division of already existing elements. Since all evidence indicates that new organic structures arise in response to external requirements, it is much more reasonable to suppose that new members of a series are produced by division of those already present. It is difficult to conceive how an externally developed element of just the right character could conveniently be present at the exact time and place it would be required, unless we are to suppose that new living units are constantly being formed of so limited a range of character that they could readily compose themselves into already operating systems. Whatever the source of the new factorial chromosomes element, it would necessarily have to respond conformably to the conditions under which it would operate. Also since the system to which it was added had already been in fully effective operation, it would have to correspond to the existence of an altered manifestation of a persisting functional complex.

While the structural development of one chromosome may with some reasonable assurance be thus conceived, it is not so easy to imagine how a whole complex, acting coöperatively, could arise. It seems to be necessary to regard each living unit of whatever degree

of complexity, as completely functional in itself, in the conditions under which it operates. Any association of elements would therefore mean, not the addition of new functions, but the altered performance of universally existent functions. The extensive and detailed genetic analysis of character behavior in Drosophila fails to show any segregation of character by groups. It would therefore be necessary to consider each chromosome and each element of it, as involved, in some measure, in the upbuilding of every character in the body. There is every evidence to show that the cellular system is one of tremendous complexity, wherein each factor plays its part by continuously interacting with all the others. This is demonstrated by the observation that the differential factors for sex affect every structure in the body. While, therefore, there seems to be no segregation of factors in the chromosomes, according to regions of the body, they are individually different and one chromosome cannot be substituted for another. It may not be without significance that this precision of operation within the system is greater and more specific in animal than in plant cells. The presence of many chromosomes in a cell instead of one, may therefore have come about in an evolutionary stage where chromosomes were being gradually integrated. Under such circumstances they would necessarily be much alike and later differentiation would result from subsequent reactions and growth. New specific numerical changes could readily come about through fragmentation or recombination. The problem of chromosome numbers accordingly presents itself somewhat in this form: (*a*) the chromosomes hold the factors necessary for the operation of the cell; (*b*) for higher forms the total number of factors is required to ensure perfect functioning; (*c*) the form of the resultant pattern of behavior depends upon the manner of interaction between the elements of the factor complex; (*d*) integration of factors into chromosomes is a method of determining the nature of concerted and sequential action of the factor complex; (*e*) rearrangement of the factors would alter the resulting pattern of characters, but not the total content.

If these considerations hold, it would follow that (*a*) a certain series of factors would be common to all animal cells. These would be necessary to condition the performance of the basic functions of life and in this respect all animals would be alike; (*b*) built upon this minimum basis are a series of evolutionary forms of increasing com-

plexity which would require, for the development of their additional characters, more factors within the chromosome; (*c*) chromosomes would therefore become increasingly more complex in their individual character and in their interrelations; (*d*) nearness of results of chromosome operation would be determined by corresponding likeness of chromosome structure and behavior; (*e*) a broad comparative study should reveal the correspondence between the structure and behavior of chromosomes and resultant somatic structures; (*f*) with enough such knowledge it should be possible to infer the causal relation between factors and characters.

The observation of constancy in the nature of chromosomes constitutes one strong support of the theory associating chromosomes with developing body characters. Constancy of results calls for constancy of means. While it is true that it might be theoretically possible to have present the total number of determining factors differently combined and still get a definite end result in development, what we actually find is that for the individual and for the species, and even for larger groups, there is a direct correspondence between chromosome characters and body characters. From group to group there is a corresponding variation in chromosomes. Unfortunately there does not yet appear any principle by which it is possible to determine the relation of a type of body variation with a corresponding form of chromosome change. The same chromosome number may appear in widely different species. There is no correspondence between phylogenetic seriation and chromosome number. These facts would indicate that, for the development of an animal body, the necessary complex of factors may be present in a wide variety of combinations, as indicated by chromosome integrations, but that, for the attainment of any specific form, the combination is necessarily constant. Through this circumstance it may be possible to determine just what it is, in the nature of chromosomes, that requires this precise organization into a complex in order to produce one specific result. Here it must be noticed that the chromosomes of a complex are marked by definite individual correspondences in size, form, structure, and behavior. This can mean only that the entire system is in a delicate state of balance.

That the members of a complex are individually different is further indicated by the fact that they definitely differ in size and that the seriation thus shown by an individual is maintained throughout the

species. Chromosome size in Drosophila corresponds with the size of the group of characters it represents—a definite evidence again of the material relation between chromosomes and characters. Usually size differences relate to length, but in Mecostethus and some other species, there is a characteristic slenderness, associated with a distinctive chromosome form. Occasionally certain regions of a chromosome are definitely thinner as in the ring chromosome of Mecostethus.

Added constancy of physical constitution appears also in the form of individual chromosomes. In part this is due to the position of the acromite but is further dependent upon the inner structure of the individual chromosome. A terminal acromite means a rod-shaped element, a median acromite, a V-shaped one, and a subterminal a J-shaped chromosome. A tetrad composed of two rods may be V-shaped, or if both ends are united it appears as a ring. Should there be an exchange of partner chromatids, multiple rings result. Similar form variations of tetrads occur also when they are composed of atelomitic elements. So far as the position of the acromite is determinative, form is constant, movement of chromatids produce less fixed results, but all are indicative of chromosome motility.

2. *In Every Mitosis This Series of Elements Reproduces Itself*

Perhaps the most striking of cellular phenomena is that of mitosis. Not only is it notable that the chromosome thus reproduces itself as a whole, but that each of its constituent elements does so most precisely also. This was early noted by Pfitzner ('82) who did not fail to inquire into its significance. It is curious to find that so few later biologists appreciate the value of this investigator's work. Only after Roux' insistence ('83) on the importance of this particulate division in relation to hereditary processes did it seize upon the imagination of biologists. Thanks largely to Weismann it has been since then the foundation stone of cytogenetics. The very fact that, of all visible cellular elements, the minute chromomeres alone so precisely reproduce themselves, points at once to their very great significance. The exact division of individual chromomeres means, therefore, an accompanying precision in the duplication of the entire series of chromosomes. Not only does this occur once, but in every one of the multitude of cell divisions which mark steps in the history of an organism it is repeated. This simple observation has an implication of profound significance—differentiation is not due, as Weismann supposed, to a

sorting out and distribution of factors or determiners in the chromosomes. There is nothing to indicate that they are in any wise altered either by loss or gain at any stage in somatic cells.

It seems apparent, therefore, that mitosis is a process for the exact perpetuation of a specific type of organization. When it is recalled that in a single organism there may be trillions of cells, that at any one time there may be innumerable individuals in a species and that the same set of conditions has obtained for perhaps millions of years, the incredible precision of the mitotic process becomes apparent. In reality, as noted, this accuracy is attained by subdividing each minute element of all the chromosomes every time a cell divides. Whenever the process goes awry in the germ cells the faulty products disappear in the test of biparental reproduction. From these considerations it does not appear strange that large taxonomic groups present essentially the same chromosome picture when any of their cells are examined. Since none of the other cell parts exhibit any such particulate accuracy in reproduction, Roux's argument for the primacy of the chromosomes in hereditary processes seems entirely justified.

3. *In Meiosis the Homologous Paternal and Maternal Controls React with Each Other as the Final Step of Fertilization* (Syngamy)

When conditions in the germ cells are considered, however, a unique and significant phenomenon is strikingly apparent. Instead of remaining separate as before, homologous chromosomes unite intimately with each other and, for a relatively long period, remain thus associated. Breeding experiments show that, as a result of this condition, an inner reorganization of the chromosomes takes place, consisting in the mutual interchange of regions containing homologous factors. It is now generally believed that this represents an actual, material interchange of inner chromosome elements at the same level of organization. Certain it is that the paired elements separate in an altered form and that this change is due to particulate, not general, reaction. Herein lies one of the main properties of sexual reproduction, which thus sets the elements of corresponding nature, derived from the two parents, in reaction against each other. It is known from the phenomenon of dominance that one of the elements may prevail over the other and prevent its effects from being manifest. To some extent, no doubt,

this feature of competition is always present whether complete dominance shows or not. The union of parental materials, begun when male-produced and female-produced cells are joined in syngamy, is carried forward when their nuclei unite into a synkaryon, is made more intimate by chromosome synapsis, and is finally consummated by the relations and activities of joined chromomeres. Such a situation provides necessarily two things: (*a*) variation in reproduction; (*b*) specific reactions between materials from two parents of somewhat unlike nature and experience. Since these conditions obtain in all higher organisms, they must be necessary for the attainment of this state, although it must be remembered that a like situation is found in the higher Protozoa. Such phenomena occur but once in the life history of each organism, but are part of the preparation of both parental elements. Since, however, they may occur at three different stages in the life history of an individual, according to the group to which it belongs, it is evident that the essence of the process concerns the chromosome mechanism. A more extensive study of the full situation in these cases may reveal the meaning of the difference in the time of the occurrence of this most significant event, but as yet it is not evident. In general the process of meiosis in the male and in the female is parallel, but in one important respect there is a difference. As a result of spermatogenesis, from each first spermatocyte four functional spermatozoa are produced. All the chromosomes are potentially continuous and in a position to carry forward their racial contribution into a new generation. In oogenesis, on the contrary, only one of the four cells is functional. Into a polocyte goes one of the homologous series of chromosomes, and thus out of possible function. So far as the series as a whole is concerned, this is a chance assortment, but for the individual chromosome it is no doubt the result of selective reaction with the entire cell. It would be quite possible in this way to effect in each case the elimination of the homologue which was less concordant with the situation represented by the egg in reaction to its environment. Extreme variation with cumulation of unfit changes could thus be prevented.

While insuring variation, meiosis also limits it. Extremes disappear because their genetic factors are incompatible within their group or with other elements of the situation. Perhaps it would be better not to stress the opposing features of variation and character continuity,

but to note that meiosis is concerned with the process of continued adaptation. It concerns the combinations of factors which meet current conditions and eliminates those which do not. It would seem, further, that somewhere in the system is a form of orientation which leads to additions, rather than to losses, so that, in the main, changes tend toward greater complexity. In effect the process of synapsis takes two corresponding sets of determiners which have been parts of two similar organisms, operating under slightly different conditions, and tries them out against each other. To the degree that they are compatible, or, as it would appear, have survived a given set of experiences with similar results, they survive as functional genetic elements. Such changes as there may be are minute and to have any phylogenetic effect must be cumulative over a long period of time. There is, within this seemingly simple act of chromosome conjugation, the basic element of biparental reproduction. Here is something unique and of infinite potentiality. It is the acid test of organic fitness, the measure of achievement in evolutionary advance, and it is applied within material limits so small as to be visible only in its larger features.

4. *Later the Homologues Separate and Segregate by Chance*

The two meiotic divisions are different from any others within the life history of an individual. They are, in reality, but one process with a single prophase. Between them they effect a recombination of all the biparental series of chromosomes and their later distribution by chance into four cells, each of which receives one chromosome of each kind. Here, obviously, is a means for securing the extreme possibility of variation when added to that provided by internal chromosome changes during meiosis. The whole process of meiosis seems to be concerned with two quite different changes in germ cell constitution. First it brings together for mutual reaction homologous elements from the two parents, and, second, it associates the altered members in chance combinations.

There was, at first, much uncertainty regarding the details of chromosome change in the meiotic divisions. There were described two cross divisions, two longitudinal divisions, and one cross and one longitudinal division. Gradually there emerged a general agreement on the last possibility and then the controversy raged over the ques-

tion of whether the first or the second division was reductional. Only later and more detailed studies demonstrated that the plane of division is a matter of individual chromosomes and not of cell generations. Moreover, the process of synapsis so alters the inner structure of individual chromosomes that segregation is a matter of chromomeres and not of entire chromosomes. Even yet it is not altogether clear why there should be two meiotic divisions. There is, however, no question but what during meiosis homologous chromosomes react upon each other, and that they then are distributed by chance, with reference to other members of the complex, into the four resulting cells. These facts are amply demonstrated by genetic experiments.

5. *Previous to Synapsis the "Individual" Material* (Cytosomic) *Is Largely Converted into the "Racial"* (Nuclear)

A complete understanding of cellular activities must, of course, embrace all the constituent parts. Because of the direct relation between nuclear structures and developmental processes, attention has naturally been focused upon the behavior of the chromosomes. It does not, notwithstanding, escape attention that the perpetuity of the chromosomes and the possibility of their action depend upon interaction between them and the remaining parts of the cell. Above all, the fact that after long periods of time, and as a result of environmental changes, the individuals of an organic group become altered in character requires some explanation in terms of current cytogenetic theories. It seems very evident that a succession of reacting individuals provide the best means to accomplish two ends: (*a*) to secure adaptation of the individual to its environment and (*b*) to carry forward an inherent developmental pattern.

Although we do not understand how it is that ontogenesis proceeds, we can follow, with understanding, its visible processes. On the other hand, because of the cyclical nature of phylogenetic changes, we are at a loss to see how a relation occurs between individuals which at once conserves a type and secures its progressive modification. By many this latter function is regarded as determined, not from within, but from without. "Variation" accordingly comes about as a result of the causative action of the environment. Now of course everything we know about organisms tells us that they exist only because of certain favorable surrounding conditions. If any one of these is lacking, life

is impossible. Should any of them fall below optimum value, an impairment of living processes results. It is therefore quite impossible to disregard the environment in an analysis of life phenomena.

Where two sets of circumstances are requisite for an operation it is impossible to regard either as exclusively causative. To understand the operation we have rather to determine the part that each plays in the effort. It is clear enough that in the living system there is a form of organization which is different from that found elsewhere, and which reacts differently from any non-living system. Also it is evident that two unlike forms of organisms, under the same set of conditions, vary in their reactions to it. There is, accordingly, something within the living system which preserves a type of reaction, and, therefore, of structure, from one generation to another and which is yet capable, as a result of many similar individual experiences, of slightly altering its type. The continuity of expression, we have learned, is due to the operation of chromosomes and their parts, and we know at the same time that these elements, during meiosis and syngamy, undergo changes which necessarily result in variation between individuals.

Variation seems to be indeterminate when narrowly viewed, but when regarded over long periods of time is clearly unidirectional. The impulse to vary thus lies clearly within the system itself. The persistence of any variation depends upon how it reacts with its environment. Perfection of reaction results from repeated similar experiences. There must, therefore, be some permanent record of these experiences—a gradual adjustment in reaction. This record we have now located in the chromosomes and so we must seek some conditions in their periodic changes which would make possible the transfer of external impressions to the chromosome mechanism. Mutations have been correlated with chromosome changes, and these may be effected by external agents such as heat, radiations, etc., but there is little to indicate that these are constructive. We seek, rather, indications of some changes which occur in the normal operations of chromosomes.

Such a suggestive situation is found in the behavior of the secondary spermatogonia of the Acrididae. These cells divide rapidly a fixed number of times, usually eight. Progressively the cytosome of each successive generation becomes smaller and smaller until in the last it is a mere shell about the nucleus. Meanwhile the chromosomes have

maintained, relatively unaltered, their original size. Clearly the substance of the cytosome has been utilized here, as nowhere else, to maintain the chromosomes. Always, of course, these nuclear elements must secure, from reaction within the cytosome, the materials for their growth, but normally this is not at the expense (or loss) of the cytosome.

Another suggestive feature of this operation is that each of the first generation of secondary spermatogonia carries with it a supply of a specific secretion from a unique somatic, or apical, cell. Since this ultimately must supply material for the upbuilding of the chromosome, it could not be without effect upon them. Commonly, with age, cells become more and more cytosomic in character. The nucleus is progressively less important in cell economy. It may be reduced to a mere remnant in superficial epithelial cells, or completely disappear in mammalian erythrocytes. As the cell becomes fixed in type the effect of the nucleus is correspondingly lessened. The changes shown by the secondary spermatogonia of the grasshopper are just the opposite of this. Starting out, the cytosome is of normal size, but in succeeding generations it is sacrificed to maintain the nuclear elements until finally it is practically exhausted, while they are of normal size. This change is invariable and results from the absence of constructive cytosomic changes between mitoses.

But any effect, to be of hereditary value, must become embedded, so to speak, in the structure of the chromosomes; must find similar expression in many individuals of the species. This speaks, not for isolated or infrequent changes in the germinal mechanism, but for a sustained and specific reaction between it and all the varied elements, organic and inorganic, with which it has to work. For that reason there is little of constructive value in sudden, isolated, and violent alterations produced experimentally in nuclear elements only without corresponding changes in the entire system. Survival changes obviously have come about very slowly, and in such a group as the Acrididae have resulted in establishing a well marked type of organism whose modifications in great numbers of species are of relatively slight range. The major effect must clearly be conservative—the maintenance of a form of organization over great stretches of time with but rare permanent alterations. It is impossible to know, however, how many unsuccessful modifications occur. What does seem to be

clear is that continuity depends upon harmonious coöperation between all the elements, internal and external, which are involved.

6. *During Synapsis While the Chromosome Pairs Act as Conjoined Units a New Cytosome Is Built Up*

During all the cell generations of an individual but one, the paternal and maternal chromosome members operate separately and, at least in some cases, in a measure of opposition to each other. In parthenogenesis a simple set of chromosomes suffices to condition normal development and in some organic groups the individual is normally haploid in its chromosome constitution. A diploid individual has always thus two sets of chromosomes in its cells, each operating to influence the development and operation of a common series of processes in slightly different ways. In the auxocytes, on the contrary, after the second gonia have completed their operation under the influence of separate sets of chromosomes and, in the Orthoptera at least, have reduced the "individual" parts of the cell—the cytosome—to almost nothing, homologous chromosomes unite intimately in synapsis and while in this condition operate to produce a new and relatively large cytosome. Since this is the beginning stage of a new generation, coming after the building up of the soma in which these haploid chromosome groups are found, the condition of the chromosome must be significant. It occurs in both male and female, but in the male the cytosome largely disappears, while in the female it becomes highly organized. The chromosome conditions of the spermatozoa and ovum correspond, but the cytosome is practically absent in the spermatozoa and strikingly differentiated in the ovum. A relatively large cytosome, in both cases, is developed while the chromosomes are synapsed and therefore operating as combined units instead of separately.

In the ovum the results of this operation are relatively permanent. The greatly enlarged cytosome can be observed to contribute directly to the development of the embryo, even to the extent of having differentiated portions distributed to specific body regions. In the production of the spermatozoa, on the contrary, the enlarged cytosome has only a temporary existence and contributes but little to the structure of the gamete. It is unreasonable to suppose that so large an expenditure of cellular energy should go for nothing and since in other respects gametogenesis in male and female is marked by essen-

tially the same processes, there must be sought some common action in the two cases. A possibility suggests itself in the converse of the relation of chromosome and cytosome in the secondary spermatogonia. There the cytosome is practically eliminated by contributing to the maintenance of the separate diploid chromosmes. In the spermatocytes, in reverse, the joined homologous chromosomes, acting as a haploid set of elements, aid in constructing a new and different cytosome, which then disappears. If the effect upon the cytosome was all that resulted, then the action would be diametrically opposite in the two cases. Should, however, the reaction upon the chromosomes be the main result, then a strong resemblance would appear in the processes of both male and female.

F. Operation of Living Systems

1. *Following Meiosis in Ovum and Sperm, Fertilization Occurs, Producing a Diploid Zygote, Thereby Uniting the Experiences of the Two Parents* (Chance Recombinations of Homologues Occur)

Meiosis is an obvious preparation of the germ cells for future activities. It is a process not comprehensible in terms of the present, but clearly looks to the future—it reduces potentialities, instead of adding to them; normally it spells extinction unless supplemented by the restoration effect of syngamy. This latter process signalizes a new beginning—the first stage in the history of another individual. The diploid germ cells, preceding meiosis, would seem, a priori, to be capable of continuous reproduction, but, instead, show a definitely limited cycle of activities. Only after they have segregated the chromosome contributions of the parents through meiosis and have had the duplex condition restored by syngamy, are they capable again of continued reproduction. The results of these contrasting operations are very evident, but the underlying causes of their occurrence are quite unknown.

To speak of "rejuvenation" as a consequence of syngamy merely describes the effect, but does not explain the cause. Evident is the fact that the newly constituted organism partakes in detail of the nature of the two parents, for from each it receives equivalent chromosomes. Also it is apparent that the maternal and paternal chromosomes remain apart from each other, sometimes even as separate

groups in gonomery; and that they may even oppose each other, as in dominance. The two parents are indeed alike in their essential nature, but each has reacted somewhat differently to its surroundings and, in some measure, its germ cells partake of this varied reaction which is finally differentially recorded in their chromosomes. To some degree each organism is a unique creation, but only to the extent that it presents a new aspect of a persisting type. Only occasionally does this variation rise to phylogenetic significance as the summation of a continued variation in one direction.

Here lies one of the central questions of biology: *"How does a persistent external impulse produce a reaction in the chromosomes of the germ cells which becomes habitual?"* Obviously if the same influences were exerted upon homologous factors of the egg and sperm it would be intensified by union in syngamy, and if this operation were continued frequently enough it would finally alter the nature of the factor. On the contrary, if a new influence were so occasional as to produce changes only now and then, the factors so altered would have no chance of survival when later tested out, in synapsis, against the prevailing type. Syngamy brings a duplicate series of controls into action to condition the changes found in development and adult functioning. The essence of this duplication, not necessary for the life of the individual, is clearly racial in its significance.

We need first to learn how these controls operate within the cell and next *how* they are involved in the coördinated activities of the entire body. There are two phases to this duplicate action (*a*) that of contrasting single function and (*b*) that of the reacting complex as a whole. These are, of course, not separable or distinct activities, but merely aspects of an organic whole. The alternative action of single factors is shown by "dominance," the influence of one factor on the whole operation, by many genetical examples. The infinite complexity of the processes of development and coördination has made them the great unsolved problem of biology, for they are indeed but two aspects of the same phenomenon. No part of the system operates alone, or free from environmental influence. Each stage is conditioned by all those that preceded it and anticipates those which follow. The only way we can conceive the origin of such a situation is to imagine that each factor arose in response to particular requirements of the system as a whole in relation to a particular environment.

The basic functions of the living system must inhere in each of its parts, and observation tells us this is true. For instance, down as far as we can see, the parts of the chromosomes reproduce themselves. Differentiation, ontogenetic and phylogenetic, does not mean the addition of new functions, but only a greater range and precision in the performance of the coördinated organic series as a whole. There is one known fact of great significance here—no matter how many cellular elements are present each bears within it the same series of racial controls which means a similar series of reactions in like sequence. Coördination, it would seem, must necessarily follow. If a similar series of tuning forks were repeated any number of times, the vibration of any one kind would set all the others of that key into vibration. Something of that nature, but infinitely more delicate and precise, must represent conditions in a living system.

Since physical systems have now been reduced to terms of electricity, it is natural that we should attempt to do the same at the organic level. It must be remembered, however, that such explanations are expressed according to the nature of the appropriate unit, and the same would have to be done with respect to the living unit. No matter, therefore, how many electrical phenomena, capable of measurement by physical means, we might encounter, it would not signify that they arose under the same conditions as would be met in a non-living system. The detection of electrical currents, varying in intensity with mental processes, does not mean that thought is merely the resultant of varying electrical potentials. The phenomena noted are doubtless the results of changes in the living system and not the causes of its operation.

But it is conceivable that with identical causal elements in every cell in an organism, arranged in like order, a change in any one of them, due to a reaction, external or internal, would provoke a response in all the others of similar character wherever found. But a change in any one of the series would influence all the other component factors and so the whole system would be affected. At any one point in the organic complex the effect would depend upon the conditions at that time and place. It is conceivable that a record of an electrical pattern might be made from such reactions, but it would not reveal the nature of the organization which produced it. This is due to the causal mechanism within the chromosome which was slowly built up

by repeated reactions—a record of experience, an embodiment of time. The thing to be noted about syngamy is that this time record is present in duplicate, so that the effect is to try out one set of experiences against another.

It thus happens that, continuously, throughout the history of the individual, in every cell, the members of the duplicate set of controls are acting in coöperation toward a common end, but the homologues, in each case, are showing slightly different influences. From the very fact that each control element is present in duplicate, there must be an infinite number of combinations of influences during reproduction of the species. An additional source of variation inhering in biparental reproduction lies in the fact, cytologically demonstrated, that in fertilization all the possible combinations of the different chromosomes occur. There are therefore three sources of variation in biparental reproduction: (*a*) chance reassortment of parts of individual chromosomes during synapsis; (*b*) chance assortment of whole chromosomes during meiosis; (*c*) chance combination of haploid complexes in syngamy.

2. *According to the Combination of Chromosomes, Male or Female, Individuals Result*

Although it was early postulated that the chromosomes contain the determining factors for the development of body characters, it was long before any definite association was established between them. The assumption, made by many, that the chromosomes of a complex are all alike discouraged any effort to seek a causal relation with developmental processes. The first attempt in this direction came when it was suggested that an identifiable chromosome, the accessory, is responsible for the determination of sex. This conclusion was reached entirely from a logical analysis of the situation. The argument ran like this: By assumption, the chromosomes carry the factors which determine body characters; in spermatogenesis, two classes of spermatozoa in equal numbers, are formed, the differential element being the accessory chromosomes; the result of the operation of this differentiating chromosome should be to produce offspring of two kinds in equal numbers; what characters do thus divide a population into such groups? The answer was obvious—clearly only sex characters.

Here there was demonstration that all chromosomes are not alike and that one of them has a recognizable relation with certain characters. Since the difference between male and female extends down to every cell in the body it is apparent that the action of the differentiator must be continuous and pervasive. Later this was shown to be true experimentally when intersexes were produced by changing the balance between the accessory chromosome and the others of the complex. The argument regarding chromosome action can now be stated in these terms: factors in the chromosomes determine the nature of cells: one recognizable chromosome can be causally associated with certain characters distinguishing sex; sex is the alternative appearance of a common series of characters, marking every cell of the body. This one chromosome operates, therefore, by modifying the action of all the others at all times. Because of the often striking nature of sex differences, this action might be regarded as peculiar, but since most hereditary differences are alternative, it probably represents the general nature of the differential action of chromosomes. It is not the mere presence of a chromosome but rather its relations in a complex that must serve as a guide to the study of its function.

3. *There Is an Early Segregation of Somatic and Germ Cells*

An individual is the temporal expression of a type of organization attained by repeated embodiments in a continuous series. Here are represented continuity and discontinuity. The individual starts as a single cell, becomes a vast multitude of cells by following a fixed series of developmental stages and finally goes out of existence. Before the final act of dissolution, however, it normally detaches certain cells which individually repeat the same cycle of changes. Continuity is material and by means of a particular kind of cell—the germ cells. In the initial stage, where the individual is unicellular, distinction between what is individual and what is racial lies only in the potentialities of the cytosome and nucleus. Only the chromosomes continue from generation to generation, and, later only those which lie in certain cells.

Since all the cells of the organism are descendants of the original single-celled zygote and since each possesses, by direct reproduction, a full complement of chromosomes, it would seem, a priori, that any cell might continue the race, but this is not found to be the case.

Sooner or later certain cells are set apart as germ cells and do not participate in the changes of differentiation. Some influence, either external or internal, alters the relation between the chromosomes and the rest of the cell so that, in the case of somatic cells, they become fixed in type and incapable of producing all types. Ascaris is an extreme instance of this. While the individual is in the two-celled stage the chromosomes of one cell break up into numerous small chromosomes in the mid-portion and cast out their distal ends. The chromosomes of the other cell remain unaltered and its descendants become definitive germ cells. It is possible that in this case, because of the parasitic habit of the worm, its somatic cells cast out the controls which are unnecessary for its simpler body development. At any rate, the process is unusual, but the fact that the germ cells retain their chromosomes unchanged is normal and significant.

Whatever process of differentiation is followed, this end is always attained. A second requisite is that the chromosomes shall not have participated in the activities of cells that have become fixed in a strongly differentiated type. Just what this means depends upon the form of organism in which the cells occur. In some, fixation is early and irreversible, in others great latitude obtains. It seems safe to say that, except in extreme cases, the chromosomes retain their potentialities, but that their form of action depends upon the cellular conditions under which they operate. After all it is only the chromosomes which are organically continuous by individual reproduction and only in them is found the record of racial experience. The discussion regarding the "continuity of the germ cells" is therefore beside the point in the light of present knowledge.

Such cellular continuity was, however, absolutely required in Weismann's scheme where the chromosomes were supposed to lose determiners progressively until finally each had but a single kind. In all higher organisms certain cells, during development, remain undifferentiated, and come to lie in a gonad, removed from active participation in the affairs of the individual in which they lie, and are later discharged from it with their chromosomes relatively unaltered. Such cells are, of course, members of a continuous line; the only question at issue in current controversies is whether they have, for a time, taken on a differentiated somatic form which they later lose. Most

of the difficulty lies practically in the uncertainty of criteria for the identification of cell types.

The release of the potentialities of the germ cells is consequent upon their removal from the inhibition of a system already advanced in its historical unfolding. They are then an anachronism, out of step with the processes of the individual, and incapable of influencing them in their course. Upon release from these conditions the germ cells may be started upon a parallel series of developmental stages. Since all the cells of an individual, somatic and germ, come from one cell, the question which naturally presents itself is: *"How does the temporal dissociation of germ and somatic cells occur?"* It is observed that in Ascaris the germ cells retain all the racial stuff—all the record of phylogeny—while the somatic cells cast off the distal portions of the chromosomes, their potential records of more recent phylogenetic experiences. In other cases (insects) germ cells are those which receive certain cytoplasmic materials. Since these inhibit immediate developmental changes, they may act by limiting the activites of the entire series of genes or of certain ones of them.

The occurrence of teratomas indicates that the inhibition of the contained germ cells in the body may in some way be removed, permitting some developmental steps. Without some such drastic change in the racial materials as occurs in Ascaris, the cells of the early generations in somatogenesis cannot differ very much, so that any of them would retain the racial potentialities and be capable of acting as germ cells. With differentiation and the consequent reaction of the racial material within formed somatic cells, modifications arise in it which become so extensive that general potentialities are lost, and with them the ability to act as controls in germ cells.

4. *Developmental Stages Follow an Inherent Pattern, Which Is a Serial Record of Racial Experience, Manifesting Itself Correspondingly in Development*

This is an observation of long standing and its explanation constitutes the big problem of biology. Why, for any one type of organism, should the pattern of cell divisions differ from that of another, particularly in view of the more fundamental fact that the main stages are much the same? There is the added fact, also, that higher forms repeat the major steps of development manifest in lower types and

then continue on beyond them with new structures. These facts indicate the existence of the following principles: (*a*) the organic record is continuous; (*b*) changes are due mainly to additions; (*c*) with time, therefore, greater complexity is attained; (*d*) such complexity is built upon earlier simplicity and does not represent an unrelated effort.

Physical continuity is visibly manifest in a line of chromosomes which, in turn, have been shown to be causally related to cellular changes which eventuate in recognizable somatic characters. When the stages of individual development are compared with the sequence of forms in geological time they are found to be, in general, parallel. Since the chromosomes are demonstrably continuous and causally related to the process of individual development, it must be assumed that in biological time new factors or determiners are added to them and that they persist. Also, since characters in individual development appear in phylogenetic order, it must follow that the factors operate to produce their distinctive effects in the order of their addition to the system. The pattern of individual development is therefore the unrolling of the racial record, which has been built up through reactions between individuals and a changing environment.

In ocean depths where environment is constant, organisms may preserve their form unchanged through geological ages. They arise, then, two great questions: (*a*) how is this record of experience made, and (*b*) how does it express itself? Development represents a progressive increase in the number and relations of parts in a functioning complex. Phylogenetic advance means, therefore, organisms of greater complexity, and since these added elements can be continued only by the presence of genetic controls, it follows that, progressively, the germinal mechanism becomes correspondingly more complex. But always new structures, to survive, must meet the requirements of the circumstances under which the organisms, of which they are a part, exist. Indeed, if phylogenetic history is properly read, these adaptations must not only be exactly fitted to the present environment, but they must, in some degree, be a continuation of past adaptive efforts. That is to say, a given change cannot occur equally well in every direction. It is only the last step in a course whose preceding steps have led up to it. The growing tip of a plant must perforce continue in the direction which has been set for it by earlier growth

stages. If there be correspondence in nature between the genetic mechanism and the results of its operation, there should be evident in it physical conditions which correspond to these phylogenetic requirements, i.e., more parts in an order, indicating progressive, unidirectional growth.

The genes have now been located definitely in the chromosomes which are linear organizations of chromomeres. It may be supposed that a continuing change in environmental conditions, extending over long periods of time, would result eventually in changes in the germinal mechanism—altered activities would evoke changes in the governing elements—a new component would be added. It would of necessity have to take its place in the existing system—it would be a new member of the linear series. The position occupied would be determined by the specific cause which brought it forth. Since new structural features are almost invariably modifications of those already existing, it is reasonable to suppose that the causative factor involved in an addition would spring from a region concerned with the control of the modified part. Presumably also it would be more distal in the series.

Development is a coördinated process during which a functional organism in a single cell condition becomes multicellular and highly complex in activity, according to a fixed pattern characteristic of the race. There are two features in this process which are fundamental: (*a*) the rapid and continued multiplication of cells, and (*b*) their ordered arrangement according to a pattern of set form and increasing complexity. What it is that causes cells to divide is yet unknown, but the process of mitosis seems designed to secure exact equivalence in cells and it usually does, but in development the same process produces successively different cells. Weismann, as noted, thought that there were differential divisions of the chromosomes by which they become successively simpler until finally they contain in each case only a single type of determiner. In other words, a given effect of a determiner is due to its *presence,* rather than to its relations in a system.

So far as we can see, mitosis actually produces exactly equivalent chromosome descendants. What a determiner accomplishes is not, therefore, a mere matter of its presence, but goes back to a question of the conditions under which it operates. In development these are

constantly changing, especially in the early stages where differences of relation are great between blastomeres. These conditions are evident and the results of their operation understandable, but why they should follow a given pattern is not so clear. Weismann did not explain how his determiners were sorted out and distributed and most efforts to account for differentiation have not contributed materially to a solution of the problem because they have failed to consider the interrelations of the elements in a very complicated system.

A factual statement of the conditions in development are briefly, these: A cell with a full duplex complement of factor controls represents the organism in its earliest, but transient stage, of development. It is, at this time, fully specific, structurally and functionally. An inherent characteristic of cells is their power, and, indeed, their necessity, to divide. Why this is so is a question of prime importance, fundamental to the solution of all biological problems. Without this power there could, of course, be no development of the single-celled ovum, but it is probably only the coördinated expression of a quality inherent in every element of the cell, certainly and visibly in the aggregates of factors, or chromomeres, of the chromosomes. As a consequence the one-celled organism becomes two-celled. These two cells, if separated, may often produce two complete organisms, showing that all the specific potentialities of the organic group are contained within them, but if they remain united, each produces only a part of the structures which constitute an organism. It is thus demonstrated that the functions of the parts of an organic system, represented by an organism here, are, in some measure, a function of their relations, as well as of their differential character. These relations are those both of space and time, perhaps primarily of time.

As the egg continues to divide, each cell receives a full complement of the chromosome elements, and, presumably of their associated ultimate factors. At the same time the cells become visibly different in two ways—in their position in the system and in their extra-nuclear materials. Each set of nuclear controls is thus obliged to operate under different conditions. Progressive variability is inherent in the system—the problem is to discover how this follows a coördinated pattern. If the assumptions are correct that control of cell operations resides in the factors, or genes, within the chromosomes, that these are successive additions to the system, and that their specific effects

occur in the order of their incorporation in the system, then it is apparent that all of the cells of the organism are fundamentally alike and that their differences are due to the order in which the factors come into operation and utilize the different materials present in the original cell which are later segregated during cleavage. It is to be noted that these materials were deposited in the ovum, not so much by its own activities, as by the action of somatic cells of the preceding generation. Light, gravity, and other physical factors are parts of the external system aiding in the differential distribution of the materials so placed in the egg.

The pattern of development is thus influenced by both internal and external factors—the ordered sequence of events and their coordinated action is governed by the identical series of controls in each cell operating throughout in an order established by their phylogenetic origin. Necessarily, a factor, if retained in the system, must come into operation at its proper time. The ontogenetic expression of the phylogenetic pattern is thus inevitable.

It is to be assumed that if a factor maintains itself in a system it must be continuously active, because a coördinated system made up of self-perpetuating members could not carry along inactive elements. If active at all it must have some influence upon the operation of the system, but that does not at all imply that the action is always the same. On the contrary, it is quite evident that at every stage in the development of a reaction system, its factors are placed under different conditions and vary accordingly in their effects. It is known from experimental studies that if a factor is prevented from acting at the normal time during development, its specic effect is altered. The conception of a time series of effects is therefore justified.

It is a mere matter of observation to note that, in development, basic or primitive structures first appear. It could not be otherwise. If the structure of the organism is due to the influence of its genic factors, then it must follow that the oldest in the series are prevailingly in operation at this time. It is equally true, however, that later additions to the series must modify the operations of the system. Thus, if the final result is the production of an organism consisting of two layers of cells, these will not have the same configurations as they would in a corresponding stage of development in another organism which proceeds in complexity much beyond this. There follows from

this four conclusions: (*a*) a factor being included in a control system must produce its specific effect from that time forward; (*b*) the form which this effect takes depends upon the constitution of the entire system; (*c*) the effect of any one factor is continuous but differs according to the stage of development reached; (*d*) at one particular time, corresponding relatively to the time when environmental reaction evolved it, it exercises a prevailing and distinctive effect upon the operation of the system. So complex is a living organism and so little is our knowledge of it, however, that any conception we may have of its manner of action can be no more than suggestive. We have also to remember that, whatever its behavior, it is always a reaction to a set of external circumstances which have conditioned the operation of such a system throughout its history, and which have, indeed, called forth its changes from preëxisting systems.

In accounting for the multitude of organic forms which now exist, there are but two alternatives—either each arose separately without reference to any other forms or they are all genetically related—the alternative is between discontinuity, on one hand, or continuity on the other. Every particle of objective evidence speaks unmistakably for continuity and that is the reasoned conclusion of all competent biologists. All organisms except the lowest are constituted of like units, or cells, and all of them perform the same limited functions. Moreover, in their individual development they all start out as single cells and become increasingly complex by cell reproduction and collective organization, according to the specific pattern in each case. For the higher type this pattern includes, at corresponding developmental stages, a repetition of the conditions in lower forms.

A high order of development is not reached by new paths but by traversing old established ways, so far as they go, and then by pushing on ahead. The only explanation of such repetition is that the experiences of the race become incorporated in its substance so that, to reach a given level of development, each individual must pass through the set of stages which lead up to it. But the individual, after having gone through this complicated process of change and expansion, disappears, but not before, in normal circumstances, it has discharged single cells, each of which is capable of producing another individual. There is material continuity between generations, provided by these single free cells. Within them somewhere lie the im-

pulses which force these cells to repeat the racial history. Such cells have been analyzed both structurally and functionally, and the concordant conclusion reached that their chromosomes are the elements within which reside the ordered forces which control the operation of the racial pattern.

Attempts to study the relations between organisms have largely been directed toward accounting for differences between them. The geneticist selects parents with contrasting characters and determines their disposition in the offspring. The taxonomist separates organisms into groups because they differ in character. Embryology is the study of progressive changes in form of the developing individual. Now and then some biologist, oppressed by emphasis upon organic differences, resulting from the application of the analytical method, proposes to study the organism as a whole, but offers no successful means of doing so. Of course, as a practical matter, the old and tried method of analysis, followed by synthesis, continues to be the most effective way of learning more about living things. Development is a history of progressively appearing differences, yet at the same time coördination is strictly maintained. The problem then is to discover the way in which a relatively simple, specifically constituted, one-celled organism becomes many celled and highly complex, while still remaining fully and specifically coördinated in its activities.

There must be found structural conditions to explain the production of differences and the maintenance of specific coördination—change and order. Cytogenetic studies have correlated the behavior of the chromosomes and of body characters in inheritance, but as yet there is no understanding of how the operation proceeds. The thought of the apparent antagonistic action of differentiation and correlation has been a handicap to a comprehension of the processes of inheritance and development. When it was shown that the controls for characters are located in the chromosomes and that they are the same in all cells, the objection was raised that with identical controls there could be no differences. Such a view totally disregards the essential nature of a living organization which is that of a reaction system. What any control accomplishes depends upon what it has to work with. There are thousands of genes in each cell—the number of vital functions are very few. Differences in the effects of genes cannot, therefore, be due to unique functions but to variations in the time,

rate, and degree with which they perform characteristic modifications of common genic functions. It is indeed conceivable that all genes are much alike, rather than strikingly different. Since they are living things they must, at all times, perform life's characteristic functions. Moreover, these must also have their peculiar specific cast. This means that there must be something in every cell of a body that is characteristically alike in order to provide the necessary unifying force. After all, the unity of an organism far exceeds its diversity of parts or unlikenesses in stage of development.

Indeed, the common characteristics of all living things are far greater than their differences, a fact we often overlook in viewing the range of structural features they present. This likeness has its basic morphological expression in the unit of which organisms are constructed—the cell. It is this fact which makes it so difficult to find the intimate causal differences between organisms, for they lie in cellular elements and the visible character range of these is not great. The structural difference between a muscle cell and a nerve cell in one organism is greater than the differences between the muscle cells of widely different organisms. Differences between cells are marked by modifications in the cytosome; the common features of all cells are latent in the nucleus, and more particularly, in its chromosomes. As yet, morphological evidence to indicate the nature of the differences between chromosomes is lacking, although they may sometimes be physiologically shown. Thus in certain hybrids the somatic cells may perform normally but at the time of stress in the germ cells—synapsis—their incompatability becomes evident.

5. *At All Times the System Operates as a Reaction System, Involving Genes, Chromomeres, Chromosomes, Nucleus, Cytosome, Cells, Organism, and Environment*

The antithesis set up between the so-called "organismal theory" and the cell theory is a false one. What else is the "organism as a whole" except the organic sum of its parts? The antithesis is given the appearance of reality by substituting for "organic sum" the conception of "arithmetical sum." The organism, whether a single cell or a multitude of cells, is a reaction system, the function of whose differentiated parts depends upon their place in the system and the time of their operation. It is no denial of this conception of coördi-

nated action at any level, to seek an explanation of the peculiar function of individual elements. Only by a knowledge of "part"-functions can an understanding of "whole" functions be gained. Analysis must precede synthesis—both are essential. No informed biologist entertains the thought of cells or chromosomes acting independently. The system of which they are elements is an integrated one in which all the members are constantly active. There can be no such thing as inert or inactive elements in the living system, for to maintain their positions, members must participate in its metabolic activities or disappear. If they are at all active they modify or influence the operation of the entire system. Even their mere presence has its effect.

It is the thought of independent or isolated action by factors which has so much vitiated conceptions of development and functioning which involve particulate factors. Weisman's idea of starting out with a germ cell having all necessary determiners and sorting them out by some unidentified force until one kind remained in each differentiated cell is an extreme instance of such views. Because of his influence, this idea has become deeply ingrained in our thought and has supplied much ammunition to those who attack particulate theories. It cannot be too strongly emphasized that in a reaction system all the elements are acting all the time. This does not, of course, mean that their operations are persistently the same. On the contrary, it is the essence of the conception that every act sets up new conditions which modify all subsequent acts.

Further, it must not be supposed that the system of factors is homogeneous or isometrical in time and space. On the contrary, the factors are arranged according to a pattern which determines all subsequent changes. This pattern is a record of previous experiences of the system—a spatial design representing temporal events. Whenever an activity occurs in the sequence which marks development, it therefore necessarily follows in the order of its addition to the series. This is the lesson which all development, ontogenetic and phylogenetic, teaches. There is, so to speak, no choice in the manner in which a factor works—it must act in the way it does normally because it became a part of the system which called for such action. If the conditions, within or without, the system are changed, the operation of the factor is correspondingly altered if the change is not fatal. It must be supposed that a factor once added to the system is retained in it

to perform its peculiar function. At the same time by its presence it will modify the operation of the earlier members of the system.

It must be realized, moreover, that the organism is not an isolated element in the cosmos, but an integral part of it. Only when certain definite and limited conditions arose was any life possible—only when even more limited and particular circumstances prevail can specific manifestations of life maintain themselves. In the higher forms, particularly in man, so well developed is this reaction to externals that even unfavorable circumstances involving depressed mental states may prove fatal. Since continued existence depends upon external reactions of a specific sort, we are logically forced to conclude that the initiation of the system depended upon the setting of such reactions. An explanation of the properties of a reaction system of this kind cannot therefore be given in terms merely of the system itself, but must also consider the external conditions which limit or render possible its operation.

For the living system there is first the fundamental property of maintenance through metabolism. There cannot be any wide difference between the method of origin and maintenance. Therefore the essential difference between the non-living and the living is the power of the latter to maintain a form of organization by transforming foreign substances into its own kind. Conditions also differ from time to time. It would seem, e.g., that the germinal vesicle stage represents a condition of stability between nucleus and cytosome, between the individual or soma, represented by the cytosome, and the germ line, represented by the chromosomes. The normal differences between nucleus and cytosome make for reactions and changes, but these are lacking in the immature egg. The large germinal vesicle apparently represents additions of cytosomic material to the nucleus, which might be the source of the balanced and relatively inert condition of the cell. Upon maturation these materials are returned to the cytosome and the reaction system is thus reëstablished. The reverse condition obtains in somatic cells under cytomorphosis, where the nucleus is often reduced in relative size and limited to one prevailing activity.

In an organism composed, at the time, of eight cells, any single cell has one fate if it remains associated with the other seven, or another if it is removed from them. If part of the group, it produces in development some particular region of the body—if separated, it

may develop into an entire organism. The one unit is, for the time being, either a single unit of a coördinated group, or a functioning organism. What any cell is, at a given moment, depends upon the circumstances in which it is placed—upon its previous history. This phenomenon of function and character depending upon place-time is one peculiar to living systems. It is also an answer to the question whether the organism creates the cells or the cells the organism. Neither is correct—the organism is a time-point cellular arrangement, one or many cells are not just units to be shuffled about by external forces, or mental concepts to be weighed by scales of formal logic—they are reaction systems whose qualities depend not only upon their intrinsic organization at the moment, but also upon what has gone before and upon the conditions under which they operate. Alter either the internal structure of a cell, its relations with other cells or with the environment, and the operation and fate of the cell are changed. It is therefore impossible to consider a cell in the abstract—it has meaning only in terms of its relations. To set up distinctions based upon whether a cell has a wall or not is also fruitless. The early definition of Schultze that "a cell is a mass of protoplasm containing a nucleus" makes no mention of cell walls, and whether they delimit cells or whether the cells are confluent is a secondary matter.

Thus, whether the organism as a whole be considered, or its parts—cells and chromosomes—it is evident that there is nothing of independence in the picture. Always the imminence of relations to other elements is to the fore. Reaction is almost another term for life, for in this way is the distinction to the non-living most strikingly shown.

6. *The Living System is Constantly Changing; and Racially, in the Main, Unidirectionally*

Of all the characteristics of living things, that of constant change is one of the most striking. Mere existence is absolutely dependent upon the continuous inflow of material from without, for maintenance and growth, and a compensatory reverse movement of waste materials to the environment. At no two instants is a living organism just the same. This is true of the whole system and of its innumerable constituent elements as well. For this reason, to liken the organism to a whirlpool or to a candle flame is a suggestive parallel—the form is maintained by a constant and limited movement of materials about a

center. In some measure such a situation is comprehensible for there is a direct and apparent relation between the materials and the continuity of the organized form. But in living things the attainment of a functionally complete state does not mean its continuity. No sooner does it reach functional completeness than it starts on a decline that soon leads to extinction.

One of the great problems of biology is to account for the constant succession of individuals. This involves the most tremendous wastage apparent in all nature. Not only does each generation of organisms go through a like series of complex and involved changes in order to become finally functioning adults, but for one such successful eventuation, innumerable germ cells are produced which fail of fruition. Then, for a few years at most, individual organisms thrive, reproduce and carry on in their particular stream of life, only at last to disappear, resolved into simple non-living molecular compounds. What is gained by this monumental waste? Why could not a persistent series of forms continue on indefinitely?

The whole lesson of biology is that the individual is not primarily important—it is the representative of a movement which finds expression in individuals progressively different. The germ line is continuous—the individual, reacting to new conditions, places the germ cells each time in somewhat different surroundings. In biparental reproduction the results of two direct experiences are tried out against each other. Those which work most accurately survive—misfits do not. Adaptation, however, may be as perfect at low levels as at high—it does not account for progress but only for survival. Progress—greater complexity, but above all greater comprehension—results from an inherent principle which is tested out by each individual.

It is, however, impossible to conceive the organism apart from its environment and this consideration does not stop with the immediate welfare of the individual, but applies also to the race—to the germ line. There is nothing more certain than that the race pursues a determinate course. The germ cells are continuous—the individuals temporary expressions of their inherent character. It is therefore apparent that the germ cells possess a somewhat unidirectional form of organization. They are going somewhere within the limits set by the environment. Change, therefore, depends upon inner organization and upon the conditions with which it works. These relations are simple enough

when only physiological needs are involved—oxygen is required for breathing, food for growth and maintenance, etc., but there are less evident, but equally important environmental relations which must be present. These are often difficult to distinguish and define. They are immaterial and operate through conscious or subconscious apperception. The higher the mental state, the greater their importance.

Viewed narrowly the relation between the organism and its environment is one of limitations and conflict. The organism fails to meet conditions imposed upon it and disappears. On the other hand only by the existence of a very limited series of physical conditions is any life possible, so that from this point of view environment nurtures organisms.

Obviously, phylogenetic changes do not result from the needs of the individual. Existence is direct proof of relative perfection in function and adaptation and with the attainment of this state, presumably there might be an unending succession of individuals of the same type. Indeed this is found in the case of certain marine Protozoa and Brachyopods. In the main, however, organisms gradually change, becoming progressively more and more complex. This is the aspect of the picture when viewed comprehensively. A study of limited populations, however, shows that between individuals of a group there are only minor differences and these are without visible trend. Excessive departure from type means extinction.

It has been customary to regard two opposing forces at work—a conservative one, heredity, which tends to maintain things as they are; and a radical one, variation, which leads away from the type. A study of reproductive processes, however, reveals a mechanism which necessarily produces offspring of a common type and at the same time ensures that each individual shall be somewhat different from every other one. Variation, therefore, is not some force opposing heredity, but is an inherent part of it. All of these conditions lie within the organism itself. At the same time it must meet the conditions under which it lives and if these change it must react accordingly. Such adjustment does not always take place and thus great numbers of species have gone out of existence. Those now living have, ipso facto, made the necessary adjustments. Over vast periods of time these adjustments are found to result in greater and greater complexity.

It is no more remarkable to find this true for the race than it is for the individual. By assumption, the first living units were the simplest; if they changed at all, it must have been toward a more complex state. There is no need to appeal to orthogenesis, an elan vital, or any other principle of perfection. Growth and differentiation are mysteries to us, but since there are obvious polarities and axes within organisms, thus revealing an inherent bias of change, it is not difficult to see that development, both ontogenetic and phylogenetic, must be prevailingly toward greater complexity.

7. *Operation of the Living System Can Be Explained Only in Terms of Purpose*

Science, in its wavering advance, returns often to the same viewpoint. In the Middle Ages, those who concerned themselves with the operations of nature were not interested in how things happened, but only in why they occurred. The purpose sought was prevailingly anthropomorphic. Then, in reaction, science became intensely mechanistic, until, in physics, the only possible advance lay in accuracy of determination to another decimal point. Quite suddenly all this was changed, until now this same subject verges strongly toward metaphysics. But for those who study living things it has never been possible to escape the implications of purpose. Indeed, there is no possible understanding of any organic structure except in terms of purpose.

"What is its function" is the first question a biologist asks when confronted with an unfamiliar part. Practical considerations and temperamental inclinations divide the students of living things into the camps of morphologists and physiologists, but sooner or later they discover that they are merely regarding the same objects from two points of view. Every minute, structural element finds its comprehension only in its functional reality. Sometimes, indeed, this purpose looks well into the future and has no immediate significance. This is strikingly manifest in the case of meiosis—a complicated series of structural changes that have no meaning in the economy of the individuals in which they occur, but are vitally significant for those of the next generation. Individuals are somewhat comprehensible mechanistic entities, but a succession of individuals, performing over and over again essentially the same processes and undergoing

the same experiences, have no meaning at all except in relation to some ultimate purpose.

Of peculiar significance here are the chromosomes and their contained genes or factors—the only materially continuous elements in an infinitely complex series of relationships. Viewed in and for themselves, these structures are incomprehensible to us; regarded as reservoirs of experience, capable of evolving like responses in future realizations, they satisfy our understanding. Of all major organic structures the human brain has least implications in its immediate present. Approached in structure by a succession of more and more complex prototypes, developed through the geological ages, it is now concerned functionally, not so much with integrating the inner activities of the body, but rather with relating the organism to its environment. By this power it somewhat controls and constructs its environment, looks into the significance of its being and begins to participate in the cosmic processes, of which it seeks understanding. As we search for purposeful meaning in the existence of parts of an organism, so we must seek for significance in the presence of organisms themselves, the coördinated aggregates of meaningful parts. So far as our measures of values go, the attainment of an instrument of comprehension and participation in the processes which have led to the production of the universe of which it is a part would seem to be an ultimate achievement.

In speaking of "purpose" in relation to the living system, nothing of the philosophical ultimate is intended. A structure is observed in the body—it has significance only because of its function in the system. Function, moreover, cannot be expressed in relations of one instant, but comprehends the entire sequence of events concerned. And these events transcend the limits of the system itself and involve the conditions under which it operates. Viewed comprehensively this associates the organism and its parts with all space and all time, however infinitesimal in effect this relation may be. Dissociation of an organism from its environment is an impossibility.

8. *It Possesses a Series of Unique Properties—Metabolism, Reproduction, Coördination, and Consciousness Being the Most Distinctive*

In all their qualities living things contrast with the non-living.

Above all, they are entirely dependent upon external circumstances. A given mass of non-living substance may remain relatively unchanged by contact with other substances over great periods of time. A living thing from instant to instant is reacting with its surroundings and drawing from them the necessities for its existence. And this is only the final response of an endless series reaching back to the beginning of its kind. Basic to all of these relations is the function of metabolism, through whose operation external, often inorganic, substances are altered specifically in character, and incorporated into the structure of the organism to replace parts lost by use or to add new ones. Undoubtedly, there are here involved many ordinary chemical and physical processes but they would not so occur apart from the organization which uses them.

Remarkable as are the processes of metabolism they are simple compared with those of reproduction. Here, in its final form, an organism of the most complex character consisting of cells in numbers so great as to be incomprehensible is able to reproduce itself through the agency of a single one of these cells, set aside for this function. Many things are involved in this incredible process, but one act is fundamental—that of cell division, which, in turn, depends upon the individual reproduction of cell parts. But it is not divisions of cells alone that is here significant—it is the order or pattern which they follow that reveals the true character of reproductive processes. Even the highest organisms begin their existence as a single cell, but these single cells are specifically organized in each case and the process of development, complicated though it may be, is merely the revelation of this condensed organization, made known by repeated cell divisions.

The ability to divide or reproduce is an inherent property in living units at all levels of organization. What is its basis? Why must an organization, once attained, be disrupted by division? Why should the products of cell division not remain of the same type and order, instead of being made subsidiary parts of a more complex system in which they take on new characters? Especially why should certain cells (germ) not undergo differential development, but retain their generalized nature while others, closely associated, specialize? These questions cannot be answered by regarding the system alone. Every

operation it performs is conditioned by the circumstances in which it finds itself—there is always a reaction to be considered.

Division is then a reaction to *what?* Division without growth, however, cannot be long continued, so there is a direct relation between these seemingly opposite functions. But why growth? A reaction system, by definition, is one of constant change in relations and character. It cannot remain in *status quo*. There inevitably follows increment or decrement from its reactions. Age of the system is here determinative and it is observed that the nuclear-cytosomic proportions (cytomorphosis) vary correspondingly. So long as the racial portion of the cell (nucleus) is ascendant, growth followed by division, occurs; when the individual part (cytosome) dominates, growth and division cease and specific function supervenes. Such relations, however, must be merely superficial expressions of profound changes in the organization of the cell as a whole, and go back to the nature of the balance between living system and environment. This is most delicate and complex. Temperature, moisture, radiations, gravity, chemical substances—all condition the existence and operation of the living system. Beyond these, especially in man, consciousness of surroundings has much to do with the nature of life processes, even to a determination of their persistence. Thus a series of processes which start with mere maintenance of the individual unit lead on to reproduction of other units and, finally, to the production of a complex type of individual, conscious of all these things and capable of altering them purposefully. It is evident that there is continuity in these conditions, and that this is but an extension of a continuity extending down to the simplest of non-living systems. Consciousness is therefore a natural and inevitable product of all these changes. It arises naturally out of increasing complexity of organization and approaches creative power, having, to some degree, reached an understanding of the conditions precedent to creation of systems. It is an end toward which all preceding conditions lead and shows the close coördination of all cosmic processes—the exclusion of chance and the rule of purpose throughout.

Consciousness at one level is an awareness of the parts within the whole. This is inherent and necessary. It is the force which coördinates the operation of the parts at every organic level. At its summa-

tion in the central nervous system of man it transcends the limits of the individual and becomes a part of the universal consciousness.

Perhaps, though, consciousness is an awareness which includes the whole, inner and outer. Organization would be an inner awareness which binds the organism or its parts into immediate correlation. Instinct would be a necessary, limited and specific reaction between the organism and its surroundings, without change of pattern from individual to individual. Instinctive action necessarily follows a given stimulus, just as does salivation at the sight of food. It is inherent in the organization and unchangeable.

Intelligence is not a fixed, individual or specific function following an unvarying pattern in the presence of a given situation. Its form of expression differs endlessly with the individual, time and place. It may not definitely be predicted either in character or degree of its expression.

The nervous system is necessarily involved in instinctive and intelligent action. In the former, the process follows a fixed path or seriation like that in a train of gears or the course of water in a drainage system. In the latter it may be altered, diverted, or stopped at any point in a manner for which no satisfactory similes may be conceived. There must, however, be some structural mechanism to account for the differences between instinctive and intelligent action. It might be supposed that instinctive action results from a train of reactions running through a permanently connected series of established course. Since there is no awareness of this process, the resulting act seems automatic, as indeed it is. There are many instinctive acts so far removed from human consciousness that they cannot be understood—such as animal migrations, involving great distances and often exact timing.

For intelligence with its varying and unpredictable processes there must be some mechanism with numerous, variable, and complex interrelations in the operation of which consciousness plays a large part.

Explanations of living systems and consciousness on the assumption that they are something apart from inorganic nature are bound to fail. It is only by inquiring how they take their place in an inclusive system that we can hope to arrive at any understanding of their nature. To assume that there are "secondary qualities" such as color, not a part of objects, like extension, can lead to nothing less than

misconceptions. To say that colors are merely a series of vibrations and that they have no existence except in an alien subjective consciousness can be done only on the assumption that we are something apart from objective nature. What is this consciousness except another expression of the reality of things? It is an attribute of our brain cells just as surely as the measurable vibrations represent a quality of a colored object. Also it may be inquired whether consciousness is a unique thing in our organization. Rather is it not an inevitable result of evolutionary development reaching down into the beginning of things and forward into undeveloped conceptions? Consciousness is an awareness, not merely of things as they seem to be now, but of the whole scheme of things. As yet it is imperfect and incomplete, not having escaped the limitations of present phylogenetic development. Indeed, its stage of completeness is a measure of such development. The endless repetition of individuals is a means for the perfection of awareness, for each individual's experiences strengthens the development of consciousness and adds to its new power.

Is consciousness something unique, as we know it in ordinary manifestations; or is it a summation of an organic function acting through all the cells of the body? Something organizes and coördinates the functions of the body. Immediate agents in this function we find in hormones, humors, etc., but the directing force, after all, is the nervous system. Still, between cells and tissues there must be some degree or kind of awareness to correlate their activities not provided for by these major agencies. Consciousness, it seems, must appear at every level of organization to eventuate at the higher level of the nervous system. If this be true, it must be an innate property of living systems —a characteristic, and highly significant function.

And this consciousness which usually means to us our knowledge of ourselves and surroundings—what is it in reality? Is it not indeed the integrating principle of life itself? Does it not exist at different levels—partly realized and partly to be developed? Does it not act to coördinate the operations of the body as well as to manifest itself in the functionings of the nerve cells—in thought and in imagination? Does it not in fact unite individuals through group consciousness? Life itself depends upon this tangible but powerful, governing force. The absolute need of individuals for each other—the difficulty or impossibility of existence apart from our fellows—is well known. And

yet we do not think of it as a biological need like that for food and other material requirements, but only as a "psychological" feeling. Remove one of the higher organisms from contact with its fellows and usually its pines away and dies. In the absence of one of its own kind it may attach itself to individuals of another species. Instances of dogs grieving themselves to death over the loss of their masters are not rare. Such matters are common knowledge and yet we do not fully recognize in them the expression of a fundamental biological principle.

Distinctions between living and non-living systems are primarily those of arrangement and relations. The arrangements in non-living systems are limited and relatively fixed—in the living, infinitely variable and mobile. The non-living are non-conscious and do not alter the cosmic systems except fortuitously; the living have risen, in man, to a conscious realization of the universe and are capable of participation in its development. Is this consciousness something outside of the cosmic elements, or is it one phase of them? If intrinsic in the scheme of things, then man incorporates in himself a considerable measure of the conceptual power which rules the cosmos.

Consciousness is generally regarded as an epiphenomenon—in some way added to the material universe without being a part of it; therefore it is not governed by the laws which rule in the phenomena which we observe about us and its operations may not be weighed, measured, or predicted.

In reality, consciousness depends upon a continuity between the brain and what lies about it. The fundamental philosophical question, after all, seems to be whether the cosmos is due to accident or to design. Since "accident" is usually a term used to cover up absence of knowledge, and since there is law and order throughout the universe, there seems every reason not to consider it the result of chance. Design usually implies a designer whom we think of as some individual, but it may well be that designer, design, and result are one. Our consciousness thus would be part of a universal consciousness.

Chance seems to us a necessity because our minds are incapable of understanding an order involving such numbers of parts and complexities of relations as obtain in the Universe in which, so far as we can analyze it, law and order rule. So when we encounter conditions beyond our understanding, our first impulse is to invoke chance

as an explanation. So far as living things are concerned they are utterly inexplicable except in terms of purpose. Often the accomplishment of this purpose is remote from the formation of a structure upon which it must depend. In reproduction and development every step is anticipatory and preparatory for later ones. It is no denial of this concept to realize that successive steps follow each other in a predetermined order because their controls were added to the system in this sequence. Such a recognition merely pushes the conception of purpose back one step from the individual to the group.

Response to varying conditions of environment does not spell chance, for the nature of the reaction is determinate and the direction of movement constant. Such variation as does exist, moreover, lies within a strictly limited range, and is inherent within the mechanism of development, and may not exceed what environmental conditions can sustain. If then, consciousness be the ultimate purpose of cosmic evolution why was it necessary to pass through all the multiple changes leading up to the production of a mechanism capable of performing this function—and to what end is its existence directed? May it be that after all, consciousness is the one reality of the universe and the different systems we recognize merely varied aspects of it? If so, nothing would lie beyond it—if not, then other, higher functions, or properties, of the evolutionary series must be anticipated, for the trend of cosmic development is always from simple to complex. There is nothing that we can conceive beyond creative power, which is inherent in consciousness, and so it seems that the only future development possible is the perfection of consciousness.

A study begun in an effort to find out how cells are organized and how they work, in particular how intracellular elements operate to produce the ordered succession of changes which mark ontogenetic development, finds that no phase of organic processes can be separated and studied alone. No appeal to inorganic phenomena, no conception of chromosome organization in terms of enzyme aggregates, or transverse and longitudinal arrangements of specific chemical substances begins to approach the true character of the problem of organic development. There is revealed a continuity throughout Nature, a progressive series of integrations of elements into units and systems terminating in the living system. Explanations of the operations of systems must, in each case, be in terms of the units of that system.

There is, in living things, a structural unit, the cell, whose operations do much to make their nature clearer, but to explain the intricate phenomena of inheritance particularly it is necessary to conceive of minute elements within the chromosomes. Such a conception has been justified by the practical results obtained through genetic analysis, and the gene takes its place in the ascending series of particles, atoms, and molecules. But no living units can be studied singly or apart from their environment—organization is a fundamental characteristic, and this represents coördinated action. Coördination requires some form of conscious relation between parts of a system. Moreover, this consciousness emerges from the confines of the organism to comprehend the circumstances under which it operates. It extends beyond the present to a consideration of past events and projects itself into the future in search of an eventual outcome. Consciousness is thus the ultimate development of evolutionary processes and associates the individual directly with the Universe as a whole. It must be invoked in the effort to understand organic processes at every level of complexity.

9. *An Understanding of the Living System Is Not Separately Attainable* (It Is a Part of the Cosmos, Derived from Other Systems, and Can Be Known Only in Relation to the Whole)

What, then, is the basic fact in the situation when the human mind attempts to conceive its origin and its relation to the cosmos? Is it not that a specific portion of the universe attempts to adjust itself consciously to the whole? How else may this be done except by that particular specific portion attuning itself in some material way to the material whole? Since, so far as we know, consciousness is a property of material substances composing the brain, then it is necessary to seek some attribute in material things, which, if raised to a sufficient level, would result in consciousness. A study of organisms reveals a common property of awareness which becomes progressively more precise and comprehensive as they increase in complexity until, in man, it leaves the present and inquires into the past and the future. Degree of advancement in consciousness seems to be measurable by the extent to which organisms can dissociate themselves from the present and associate themselves with all time.

The nature of organisms, we find, depends upon their past history.

What they are today is determined by the experiences of their ancestors. In some material way this experience in time becomes incorporate into their actual substances and becomes a part of them. In this measure they are four dimensional things and apparently in a progressive way time becomes a more and more important element of their being. It may not be inconceivable that ultimately they should become one with time. In such a form this statement is, of course, purely metaphysical. There are concrete facts, however, which show that experience becomes definitely impressed upon living things, changing their nature, even that part of it which we speak of as mental.

A direct and striking instance of this general effect is afforded by the action of radiations upon the genetic expression of group characters. Changes, which under ordinary conditions occur at a low rate are speeded up many times. Radiations, apparently material things, acting upon living matter, change its relation to time by hastening organic change. But radiations themselves represent a time effect, through which, e.g., uranium finally becomes lead. It is in one sense as if this slow effect in inorganic matter is passed on to living substance at an accelerated rate. Indeed, in many ways this is a characteristic relation for often an extremely slow process in the non-living appears in similar guise in the living, but in a much more highly developed and rapid expression.

It is quite impossible to conceive living things apart from time—it is their real essence. From instant to instant they are different entities. As individuals they develop from a state of relative simplicity to one of great complexity as active centers. They transform non-living matter into living and again reconvert it; every instant they react to external conditions and especially in mass, and by this reaction change the nature of all things around them. As the individuals change, so likewise do the assemblages which they constitute, and the long time view of this process shows progressive change from the simple to the complex. The essence of this complexity lies in the inherent capacity which it grants for greater speed, precision, and control in reactions.

When the nature of the mechanism which governs the relation in time of organic units is inquired into, it is found to consist of discrete units, of apparently definite number in each case, which through

their specific action and interaction within a limited and integrated series of similar units, connect an order of processes of a definite pattern in time. This time pattern is in some way directly related to a space pattern of a determinate order.

Thus a limited and practical investigation, begun to discover the relation between the conditions of an organism in the one-celled stage and as it appears in its final state of development, has become involved finally in fundamental cosmical problems. This outcome was not foreseen in the beginning, but as the analysis proceeded it was found impossible to stop at any part way stage even for the limited purpose in mind. What we know as vital processes cannot, on the one hand, be dissociated from other phenomena, nor can they be identified directly with them. They rise above the level of inorganic processes, but, at the same time are dependent upon them. Progress in an understanding of life in its varied aspects depends upon a clear distinction between what is inherent at the vital level and what is contributed from lower levels of organization. Neither by abandoning the field through an effort to restrict conceptions to the inorganic type of phenomena, nor by confessing failure in appeals to hypothetical forces of unknown character can understanding be reached. For my own guidance in thinking of these matters I considered the situation broadly as it appeared to me. In the hope that my friends, who urged me to make the outline more widely available, are correct in the view that it may be useful to others, I have prepared it for publication. Whether this particular formulation of ideas provides a basis for further advance in knowledge or not, it is perfectly obvious that until we have such a guide, progress in understanding of differentiation and development will be slow and uncertain.

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FOUR YEARS' EXPERIENCE WITH P-DIMETHYLAMINO-AZOBENZENE DIETS AND RAT HEPATOMAS*

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In 1937 one of us (Medes) was studying the enzymes obtained from rat's liver which were effective in the various transformations from completely reduced to oxidized sulfur. The results of these experiments have been published elsewhere (1, 2) and showed that a number of separable enzymes are concerned in the step by step transformations of these compounds (3). It was desired then to study in comparison the enzymes, if any, accomplishing the same results in cancers arising in the liver. The feeding of p-dimethylaminoazobenzene, "butter yellow," was begun as a means of obtaining this experimental material. At that time Kinosita, visiting the Institute, gave a detailed account of his work and made several recommendations supplementing his publication of 1937 (4). He stated that the cancers of the liver could be procured more rapidly by substituting polished rice for the unpolished in the basal diet of rats. Unpolished rice gave rise to tumors in an average of about 70 days but those rats fed the polished variety exhibited a latent period of approximately 40 days. Kinosita further stated that the substance in the polishings responsible for the inhibition was probably a vitamin of the *B* group although it did not appear to be any of the factors which were known at that time.

Since 1939 much has been learned of the relationships of these factors, but the experiences obtained in this laboratory are a sort of history of these advances, so may be of interest to others. They are therefore recorded here. It is emphasized again that the purpose of the feeding was not to study the development of the tumors, but merely to procure them or to work out conditions for obtaining them for enzyme studies.

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Experimental

A. Thirty-six rats of about four weeks of age (50 to 60 grams) were fed butter yellow for 75 days. The basal diet consisted of 1000 grams of finely broken polished rice, plus 20 grams of 1 per cent butter yellow in olive oil administered for 5 days, 2 per cent butter yellow for 7 days, and 3 per cent butter yellow for 50 days. The supplements of fresh vegetables listed by Kinosita were adhered to. The food consumption was measured daily to ensure that during the period of 50 days the rats obtained at least the 180 mg. of butter yellow which Kinosita had said (p. 683) were essential for the production of hepatomas. At the close of that time, the animals were vigorous and lively, and since, in contrast to those described by him, they had shown no signs of toxicity, the special diet was continued for an additional 13 days with the oil containing 4 per cent butter yellow.

Kinosita stated (p. 685) that longer feeding speeds the production of hepatomas and results in a larger number of small tumors. As we were desirous of obtaining large single tumors, the rats were removed from the experimental diet after the 75 days, contrasting with the usual method of continuing the butter yellow until death. The rats were then returned to the laboratory stock diet of checkers, liver, fish, cheese, yeast, fresh vegetables, milk, eggs, Cygon, and Navitol. They were maintained on this regime until they were one year old, at which time they were killed and autopsied. No hepatomas were found upon gross examination. Microscopic sections of the liver revealed no evidence of even "precancerous" lesions in any of the animals. Thanks are expressed to Dr. J. E. Edwards of the National Cancer Institute for separately examining these slides.

At that time the experience of American investigators with the difficulty of producing liver cancers in well-fed rats had not been recorded. One suggestion as to the cause of our failure to obtain hepatomas as did other workers was that we had substituted polished for the brown rice commonly employed. Consequently another series of experiments was carried out taking this into account.

B. Fifteen rats, 9 males and 6 females, were used to repeat the butter yellow feeding experiment described above with all conditions the same except that unpolished rice was substituted for polished and the Kinosita supplements were measured daily and held strictly to standardized amounts. The animals were sacrificed 130 days after

the end of the 75-day period on butter yellow, a shorter interval than in *A*. They were then 233 days of age. Of the 15 rats, 5 had developed hepatomas. The other livers showed changes varying from mild degeneration to early cirrhosis. Under the altered conditions of this experiment, including notably a regulated vitamin intake, the substitution of unpolished for polished rice permitted a larger percentage of tumors to develop.

C. More data as to the rôle of the vitamins biotin and riboflavin having accumulated in the literature, the next group, consisting of 10 rats, was given an increased amount of biotin. The same basal diet of polished rice and butter yellow in olive oil was fed for 75 days. Our rats exhibited none of the "toxic" symptoms mentioned by Kinosita, although often consuming far more than the required amount of butter yellow for the given time. Kinosita's supplementary diet was now abandoned as being insufficiently controlled as to vitamin content for our animals. One gram of fresh carrot and two drops of Navitol were administered daily and 200 micrograms of biotin were added to 1000 grams of rice. Other vitamins of the *B* group were administered in the following amounts per 1000 grams of diet.

Thiamin	4 mg.	Pantothenic acid	50 mg.
Pyridoxine	4 mg.	Riboflavin	4 mg.
Nicotinic acid	50 mg.	Choline chloride	500 mg.

These rats, sacrificed 147 days after return to the regular stock diet, were 245 days old. One had a hepatoma; one had a normal liver; the others showed varying degrees of cloudy swelling and fatty degeneration but no necrosis nor cirrhosis.

D and *E*. About this time Laurence's note as to the possible part played by avidin in spontaneous regressions in malignancy appeared in the literature (5). The following experiment was made with two groups of rats to test the effect of varying the ratio of biotin to avidin under our conditions. (*D*) Nine rats were maintained for 75 days on a diet similar to that above (*C*) except that biotin was omitted and 40 grams of powdered egg white were added to 1000 grams of polished rice. This was the only group of rats that at the end of the 75-day diet was obviously in poor condition. They were weak, listless and partly bald and were consuming very little of their food, in marked contrast to the good condition and very aggressive behavior

of the rats that received biotin. The animals were killed after a further 135 days. One hepatoma and one normal liver were found, and the remainder showed degeneration. With the other group, consisting of 8 rats, the same regime was maintained except that 500 μg. of biotin were administered per 1000 grams of rice in addition to the 40 grams of dried egg albumen. The animals were killed after 135 days on the normal diet following the butter yellow feeding. No hepatomas were found on autopsy but all except one showed degeneration. It must be recalled that these rats were ingesting 4 mg. of riboflavin per 1060 g. of diet, representing an intake of about 32 μg. for each 8 grams of food. In addition they were obtaining 1 gm. of fresh carrots daily. As 8 grams is approximately the amount of food eaten by our rats under the conditions of these experiments, they were receiving riboflavin on a level capable of exerting some protective action.

F and *G*. Finally, to decrease the riboflavin effect, another experiment was performed with 9 rats, *F*, from which all of the crystalline vitamins were withheld except biotin. A similar group of 9 animals, *G*, received the supplementary vitamins as well as the biotin. Among the rats that received the supplementary vitamins no hepatomas were found. In the group without supplementary vitamins, 4 hepatomas developed; all other livers were degenerated. This was the highest incidence of hepatomas, being 50 per cent, as one rat was always killed for study on the 75th day.

The biotin and riboflavin contents of the various diets are estimated in the accompanying table (Table 1). These estimations are necessarily rough, as the daily food intake was not measured and 8 grams was used in the computations as an average figure—an amount open to wide fluctuations. The riboflavin and biotin values were generously supplied by Dr. Burk and Dr. Robertson of the National Cancer Institute. This inactivation of 2.2 micrograms of biotin by 1 gram of dry egg white is the figure suggested by Eakin, Snell, and Williams (6). As may be seen from the chart, no consistent relationship can be deduced between the biotin content of the diet and the incidence of tumors, whereas a high negative correlation is exhibited between riboflavin intake and hepatomas. Evidently the animals in Groups *C*, *D*, *E*, and *G* had considerable protection, whereas it was much reduced or was about absent in Groups *B* and *F*. It may be

TABLE 1

DAILY INTAKE OF BIOTIN AND RIBOFLAVIN PER RAT ESTIMATED ON AN AVERAGE CONSUMPTION OF 8 GMS. OF FOOD

The following figures were used for calculating the vitamin content of dry weight of food:

Biotin

Carrots	0.15 gamma*
Polished rice	0.04 " **
Brown rice	0.1 " **
Egg white	1.0 " *

Riboflavin

Carrots	0.75 gamma per 1 gm. (fresh weight)***
Brown Rice	0.6 " **
Polished rice	0.4 " **

	B	*C*	*D*	*E*	*F*	*G*
Biotin	0.82	1.94	0.66	4.66	4.66	4.66
Riboflavin	5.55	35.95	35.95	35.95	3.95	35.95
No. rats	14	9	8	7	8	8
No. with tumors	5	1	1	0	4	0

*From table supplied by Burk, Spangler, Winzler.
**We are indebted to Dr. Dean Burk for obtaining these values from Dr. Robertson of the National Cancer Institute.
***Heinz tables, 1940.

noted that the group, *F*, which received the lowest riboflavin and the highest biotin supplements, showed the greatest tumor incidence.

Since the food consumption was not measured, too great reliance cannot be placed on the absolute amounts estimated for the daily intake of the two vitamins under question. Nevertheless a rough comparison with the results of other investigators may well be made.

Nakahara (7), using a brown rice diet, found that a liver eluate preparation which supplied 20 micrograms of riboflavin daily offered no protection.

Miller and co-workers (8) showed that casein at levels of 18 to 40 per cent offered partial protection against butter yellow. Dried liver, replacing part of the casein, was still more effective. About 25 micrograms of riboflavin per day increased the protection.

Kensler and collaborators (9) obtained an incidence of 96 per cent of liver cancers when rats were given butter yellow added to the usual rice diet. With an additional supplement of 200 micrograms of riboflavin or 2 grams of casein daily, a slight protection was afforded (70 to 80 per cent of cancers) but with both the riboflavin and the casein added the incidence fell to 7 per cent.

Du Vigneaud and co-workers (10), in one experiment, used 82 per cent of brown rice, with 18 per cent of casein supplementing the protein portion of their diet. The crystalline vitamins they employed contained 1 mg. per cent of riboflavin, or 80 micrograms daily on the basis of an estimated food consumption of 8 grams. The feeding of butter yellow resulted in one hepatoma among 9 rats, but addition of biotin at levels ranging from 0.3 to one microgram daily increased the percentage of liver tumors to 25 and 50. Even more striking results were obtained when 15 per cent casein and 10 per cent egg white supplied the protein portion of the diet, the latter at the same time reducing the effective biotin level. Addition of 0.5 mg. per cent of riboflavin for the first 45 days, followed by 2 per cent thereafter, resulted in no tumors in 5 animals, whereas further addition of 2.0 micrograms of biotin daily increased the incidence to 3 out of 5.

In the experiments recorded here, the basal diet consisted in all but one instance of polished rice, as contrasted with the more efficient casein used in much of the other work. Riboflavin, at the level of 4 mg. per 1000 grams of diet, or an intake of about 36 micrograms daily, afforded nearly complete protection when the biotin supplement was reduced to less than 0.5 micrograms daily. When no riboflavin was administered and the biotin level was about 4 micrograms per day, the incidence rose to 50 per cent. This represents a higher intake of biotin than in most of the experiments reviewed above, and a level of riboflavin agreeing well with that employed by all of them except the du Vigneaud group. The high riboflavin supplements used by the latter workers offered approximately the same protection afforded by the 36 micrograms supplied in the experiments recorded here. No explanation is available for the discrepancy. Unfortunately, data as to the comparative food consumptions are not available.

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THE NUCLEAR REACTION TO SH OF EXCISED SALIVARY GLAND CELLS OF SECOND AND EARLY THIRD INSTAR LARVAE OF DROSOPHILA MELANOGASTER*

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A. INTRODUCTION

When Painter (22) and Heitz and Bauer (15) rediscovered the giant chromosomes of the salivary gland cells of Diptera larvae, and suggested possible interpretations of vital processes in terms of these gland-cell nuclei, an entire new field for physiological, chemical, and genetic experimentation was opened up.

Since our immediate interest is centered in the chemical factors for growth and development, it occurred to us that these cells might serve as useful test material for an approach to the problem of the chemistry of cell multiplication; not only because changes in their chromosomes can be followed with relative ease; but also because there is a general belief that these cells do not increase in number after the larvae are hatched (3, 19, 23, 28, 29).

The literature affords some pertinent data which can be grouped as follows: (*a*) chromosome make-up, (*b*) chromosome composition, (*c*) growth expressions exhibited by salivary gland cells.

1. *Chromosome Make-Up*

Using isolation and micro-dissection technics, Buck (4) concluded that these chromosomes can withstand 100 per cent elongation without apparent deformation. In addition they resist shredding and thus seem to be rather solid structures. Buck has suggested that lipoid may play a rôle in the fabric of the chromosomes, for when they are treated with ether, elasticity is lost and they can be stretched almost indefinitely.

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Painter (23) found that when the nucleic acid components have been digested away with alkali, the chromatic material remains as chromonemata upon which the chromomeres are located. From these and similar observations of many different strains he came to the following conclusions regarding the make-up of Drosophila and other related salivary-gland chromosomes.

He considers the mature structures as multiple thread or polytene objects developed from the original four chromatids of a synapsed pair. The bands are taken to be made up of chromomeres, closely oppressed, the individual components of which cannot be seen. The connecting threads, or chromonemata, are comparable to cables; being compounded of single chromonemata. The increase in size of the salivary-gland chromosomes over that of other larval cells is assumed to be brought about by a reduplication without visible division of the parts. In addition there is said to be a gradual lengthening resulting from uncoiling of the components.

He assumes that the chromosomes pass through changes equivalent to the resting phase, prophase, metaphase, and so on of the usual cell; or a sort of endomitosis. It follows from this assumption that the endomitotic processes of reduplication are expressions of physical and chemical changes within the chromosomes; and that we therefore may expect to find no two cells behaving alike when exposed to chemical or physical agents.

Metz (21), on the other hand, does not wholly agree with these interpretations. He does not believe that the bands represent chromomeres; but rather differentiated areas of chromonemata. Nor does he believe that the chromosomes pass through any stages comparable to those of normal mitosis; but that they remain in the resting stage throughout their life. Naturally if this is the case, the number of granules in the band cannot be taken as an index of the number of multiplications through which the original chromosomes are assumed to have passed.

From this point of view the size increase of the chromosomes would not be a "reduplication" in the sense used by Painter (23); but simply an expression of anabolic growth or constructive substance increase (12).

2. *Chromosome Composition*

In addition to the demonstration of the presence of lipoid in these

chromosomes (4), Schultz (25) has come to the conclusion that there are " . . . two and possibly three types of protein; a protein resembling serum globulins, one distinctly basic in character, and in addition a protamine-like component."

Caspersson (5) by ultraviolet studies of absorption, and Mazia (20) by enzymatic digestion studies, have provided data which indicate that thymonucleic acid and protein are systematically arranged throughout the chromosomes. The continuous substratum seems to be composed of a histone-like protein, while the bands contain a protamine-like substance.

3. *Growth Expressions Exhibited by Salivary-Gland Cells*

During larval growth the increase in the size of the organ seems to be a matter of cell increase in size rather than one of cell increase in number (3, 28, 29). Absence of mitotic figures were the criteria in most cases; but Makino (19) actually counted all the cells on one side of the flattened gland of *D. virilis*. From such counts he estimated the total number. He found an average of 113 in the first instar, 114 in the second, and 111 in the third.

Frolova (7, 8) claims that as the larvae grow, the nuclei and chromosomes of all somatic cells grow also. "Their diameter gradually increases, that is growth of chromosomes sets in. After this the number of chromonemata is doubled and therefore the diameter of the chromosomes also becomes doubled." He bases his conclusions on camera lucida drawings of cells from larvae of different ages. He does not attempt to explain the second duplication which he says occurs later. He assumes all the cells to remain in the resting stage and thus agrees with Makino (19).

Measurements made by Ross (24) showed that the salivary glands of mature first instar larvae may be 65 mu long and 20 mu wide; while 60 hours later at the end of the third instar they may average 1100 mu in length and 100 mu in width. During this period the nuclei may increase from an original diameter of 2.4 mu to one of 20 or 25 mu. Similarly the chromosomes increase, either by "reduplication" and/or anabolic growth from one to two thousand-fold (27).

Practically no direct observations have been made concerning the factors involved in the growth of these glands or their cells. Colchicine gave no growth but only osmotic effects (2).

The marked size increase in these structures without apparent increase in cell number suggests that here is exceptional material for inquiry into the chemical factors specifically concerned in cytoplasmic and nuclear substance synthesis.

Such a study might well help answer the question as to whether this growth of the chromosomes is simply constructive substance increase, or whether there is combined therewith a "reduplication" in the endomitotic sense.

But this is for future work.

What we are interested in just at present is the claim that these cells do not proliferate, and whether or not they can be brought to express this activity by exposure to an appropriate chemical stimulus.

Since it has been found that sulfhydryl (SH) is a naturally occurring chemical stimulus essential to growth by increase in cell number (9); and since it is evident that this chemical group fulfills all the requirements demanded of a specific determinant of proliferation (12); it is plain that an investigation of the reaction of salivary-gland cells of Drosophila larvae to sulfhydryl is in order.

Regardless of outcome such an inquiry should yield, as it has, data of value to a chemical concept of growth.

B. Material and Methods

The wild strain of *Drosophila melanogaster* known as the Swedish-b obtained from Villa Alba Laboratories of Delphi, Indiana, was used. The flies were raised on a banana-agar medium well supplied with fresh baker's yeast. They were kept in a constant-temperature incubator at 25° C.

Larvae were reared from eggs collected over a two-hour laying period and fed banana-agar medium with fresh yeast. It seemed desirable to study cells actively increasing in size rather than cells from mature glands. Nuclei of first instar larvae are too small to see easily; but those of second and early third instars may be readily inspected. We therefore used larvae taken after from 32 to 72 hours beyond the median hour of egg laying. Such are from 2.3 mm. to 4.5 mm. long. The length of the glands varied from 300 to 700 micra. An average size for the cells and nuclei was not determined since this varies greatly depending upon the amount of the secretory granules in the cells and the amount of secretion in the lumen of the gland.

Workers with these glands have found that when they are dissected into Ringer's solution or blood serum there is a marked shrinkage of the nuclei. Stern (26) however was able to keep Drosophila testes

alive and apparently healthy in dilute sea-water. After some preliminary trials it was found that a one-to-four dilution of this medium kept the cells osmotically active and apparently "alive" over a period of several hours, if the dilution was carefully made and evaporation prevented. In some cases the glands, cells, and nuclei appeared in good shape after 12 hours in such a culture medium.

According to Buck (4), osmotic activity is a prime characteristic of living nuclei.

The pH of the diluted sea-water averaged 7.5 as tested with a Coleman apparatus. Tests were made in dilute sea-water the pH of which had been adjusted to 5.6, 6.0, and 7.0 with HCl. Glands so exposed showed many irregularities in cell outline and protoplasmic structure. Those cultured in sea-water at pH 7.5 showed no such deviations. The satisfactory results on the alkaline side are probably due to the fact that *D. melanogaster* blood has a pH of 7.1 (1).

The sea-water was collected daily, filtered, and heated to boiling to destroy micro-organisms. Fresh dilutions were made each day. These must be most carefully made for the nuclei are very sensitive. If the culture solution is too concentrated they immediately shrink; if too dilute they become so hydrated that they appear optically empty. It is just this sensitivity to osmotic changes that makes them so useful in physiological studies. *Aliquots of the same sea-water were used for test and control culture solutions.*

The source of SH in the test solutions was a specially purified para-thiocresol. It had been previously determined by Wilson (30) that this compound in 1×10^{-7} concentration of sulfur as SH stimulates length growth of the larvae and is non-toxic. So this concentration was used throughout these experiments. Preliminary trials showed that a stronger concentration gave cells with protoplasmic abnormalities.

The care with which sulfhydryl compounds must be used has been emphasized and re-emphasized (13). But the need for meticulous technic cannot be over-emphasized when dealing with this extremely sensitive chemical group. All slides, culture dishes, and working surfaces were kept scrupulously clean, and no other experiments were performed in the portion of the laboratory used for these investigations. No crystals of para-thiocresol were used which had become even slightly tinged with yellow because of exposure to air.

Stock solutions were made fresh each day by adding 0.039 gram of the compound to 10 cc. of 95 per cent ethanol. A graduated pipette was used to add the requisite aliquot of the stock solution to the diluted sea-water in each experiment. Stock solutions were kept in the refrigerator in the dark. Experiments were not performed on hot days.

The glands from two larvae of the same age were dissected out in a depression slide under a binocular microscope. Care was taken to preserve the ducts intact. Fat cells were cleared away and the common duct was left attached to the mouth armature. One gland was covered with test solution; the other with the same dilute sea-water to which ethanol had been added in concentration equivalent to that of the test culture solution.

The slides so prepared were placed on wet filter-paper in flat petrie dishes and left at room temperature for designated periods. Illumination was the same for test and control. Solutions were changed hourly when experiments were protracted. Often peristalsis of a part of the oesophagus at the end of the period of culture could be used as an index of vitality.

In the beginning the glands were directly examined under the binocular microscope. Since it was found more satisfactory to subject them to preliminary staining with an 0.05 per cent solution of the vital dye Janus green B, this procedure was generally followed thereafter. When properly stained they were transferred with a capillary pipette to a slide and gently covered with a cover-slip supported on broken pieces of cover-slips. Only experience can determine the best staining for clear observation.

The specimens were studied under a compound Zeiss microscope using 15 x ocular and 40 objective. Measurements of cell size were made with an ocular micrometer.

After the technics were perfected 57 sets of glands were dissected out, stained, and studied. In all these there was no injury to the gland nor did the cells appear abnormal in any way. The test and control glands were simultaneously cultured for the same length of time in each experiment. The length of experiment in the different experiments ranged from 15 minutes to 4 hours. At the end of the stated period the tests and controls were compared with particular attention being given to the nuclei and their contents.

C. Results

The most dramatic difference between test and control specimens was the translucency and size of the nucleus. Almost without exception the nuclei of the glands subjected to SH were much larger than those of the controls, and they were distinctly paler and more watery looking.

The measurements of cell and nuclear diameters and the ratios

TABLE 1

Nuclear Reaction of Salivary Gland Cells to SH in Dilute (1:4) Sea-water Culture

Duration of exposure	Instar	Staining intensity	Nuclear translucency	Diameter Cell/Nucleus Test	Diameter Cell/Nucleus Cont.	Diffuse nucleolus
15 min.	3		+			+
15 min.	3	=	+	2.2	2.9	=
15 min.	2	=	+	2.1	2.4	+
15 min.	2	=	+	2.3	2.8	+
30 min.	2	+	+	2.5		+
30 min.	3	+	+	2.2	2.7	+
30 min.	3	+				+
30 min.	2	=	=	2.5	2.5	=
30 min.	3	+	=	1.8		+
1 hr.	3	+	+	2.1	1.8	=
1 hr.	3	+	+	1.7	3.3	+
1 hr.	3	=	=	2.4	2.0	+
1 hr.	3	+	+	1.8	2.8	+
1 hr.	3	+	+	2.0	2.5	+
1 hr.	3	+	+	2.3	3.0	+
1 hr.	2	+	+	2.6		=
1 hr.	2	+	+	1.7	2.8	=
1 hr.	2	+	+	2.0	2.5	+
1 hr.	2	=	=	2.8	3.5	=
1 hr.	2	+	+	2.3	3.0	+
1 hr.	2	−	+	2.2	2.5	+
2 hrs.	2	+				+
2 hrs.	2	+				+
2 hrs.	3	+	+			+
2 hrs.	2	+	−	3.3	2.2	+
2 hrs.	2	+	=	2.4	2.8	=
4 hrs.	3	+	+	2.2	2.8	=
4 hrs.	3	+	+	2.0	2.5	+
4 hrs.	3	=	+	2.5	2.5	=
4 hrs.	3	+	+	2.2	2.9	+
4 hrs.	3	+	=	2.8	3.5	+
4 hrs.	2	=	=	1.5	2.0	=
4 hrs.	2	+	=	3.0	3.3	=
4 hrs.	2	=	+	2.5	2.6	=
4 hrs.	2	+	+	2.5	4.0	+
4 hrs.	2	=	+	3.0	4.0	+

between them show conclusively that not only an absolute but also a relative increase in size of the nucleus took place in the tests. The latter values are given in Table 1. Estimates of the difference in translucency are also given in Table 1 in terms of "more-or-less".

This absolute and relative increase in nuclear volume, when taken together with the increased translucency of the nuclear sap is pretty good evidence that nuclear endosmosis has taken place in the SH-containing cultures. The interpretation will be given in the discussion.

Often throughout the summer some nuclei would be found in a shrunken condition at the end of the period of observation. Such would exhibit irregular outlines but no crenelation. They were of course discarded. Sometimes the nuclei of controls would shrink while those of the tests would hold their turgidity. In only one case did the reverse occur. Obviously this shrinkage was a result of exosmosis. Why it did not occur in the tests will be shortly evident.

Notable differences between test and control specimens also obtained with respect to the chromatin. Pretty generally this took the stain more intensely in the SH-exposed material. The occurrence of this greater stainability is recorded in Table 1.

According to Frolova (7, 8) the typical chromosome picture in these cells shows a chromo-center from which radiate the homologous chromosomes over the surface of the nucleus. The large nucleolus is located in the center of the oval or spherical nucleus.

In our preparations the radiating spireme-like nature of the chromosomes could sometimes be detected; but in many cases the cells of the controls were more homogeneous and showed little evidence of organized structures. Longer staining of the controls only resulted in overstaining rather than differentiation of the structures.

The test nuclei differed from the control in two respects. In the first and less frequent the chromatin became definitely segregated in the center of the enlarged nucleus as a more or less closely knit prophase-like spireme. In the second and more usual the chromatin segregated at the periphery in fragmentary coarse particles of varied size.

The nucleolus was less pronounced in the stained test nucleus. Careful examination suggested that this was not due to a lesser stainability as such; but to the fact that the structure was more diffuse as if undergoing partial disintegration. This is recorded in Table 1.

As far as the cytosome is concerned there was very little difference if any between test and control cells and what differences did occur were inconsistent and non-specific. Infrequently in both sets the cytoplasm of one cell would take the dye more readily than another.

Secretion granules and vacuoles increase in number in the older third instar larvae as might be expected in view of the increase of secretory products in the duct with developmental progression. For the most part, however, in the glands studied the cytoplasm was reticular with very small vacuoles and was quite uniform in appearance whether in tests or controls.

In general then it was the nucleus which gave visual evidence of reaction to SH and not the cytoplasm. Furthermore, and this seems significant, while the nuclei of the control cells were more or less uniform in appearance, those of the tests showed considerable variation in all the aspects described in preceding paragraphs. It is as if various stages of activity were taking place in the test nuclei while the control were essentially quiescent.

All the hereinbefore differences capable of being recorded are so shown in the accompanying table arranged according to length of exposure to SH. A plus mark indicates the reaction was greater in test than control; a minus that it was less; and an equal that no difference was determinable.

D. Discussion

No evidence whatsoever was obtained that cell increase in number was induced by SH during the course of these experiments even though 10 trials were run for four-hour periods of exposure to the stimulant.

While it could be that the unnatural conditions prevented such or that insufficient time was allowed, to use these as excuse for failure to induce proliferation would seem to strain credulity beyond endurance.

On the other hand most any one of the changes in nuclear chromatin induced by exposure to SH might, if taken by itself, be justly considered as a mark of pre-proliferation activity, since such are seen in cells which do go on to divide.

Thus, according to Lewis and Bremer (18) "*The first indication of approaching division is a change in the chromatin which becomes gathered into* fewer and *coarser granules and takes a deeper stain.*" This

phenomenon occurred frequently in our experiments. ". . . *The chromatin granules come to be arranged in long convoluted threads.*" This occasionally happened here. ". . . *The nucleoli break up into smaller bodies. . . .*" This was frequently observed in the SH-treated cells.

If it be granted that these phenomena were here pre-divisional as they are acceptably so elsewhere they are evidence that here as elsewhere SH acts to forward some process or processes concerned in cell increase in number.

Why these cells did not go on to complete division must at present be a matter for speculation.

Now it is a commonly observed fact that proliferation activity slows down as cell differentiation progresses. This has given rise to the belief that the more differentiated the cell the less its potentiality for division. Since the salivary gland cells of Drosophila do not increase in number despite the marked changes the rest of the organism undergoes as it passes from one instar to the next; and since these cells are sufficiently specialized to produce abundant secretion; it could be that they have differentiated beyond the stage where proliferation is possible.

To be a bit more precise; it can be reasonably assumed at present that the salivary gland cell-as-a-whole of the second and third instar larvae has gone so far along its road to chemical specialization that the chemical groups necessary for expression of its division activity have either been tied up by the molecular rearrangements of specialization or otherwise rendered unavailable. Therefore these cells are unable to react by increase in number even though presented with a naturally occurring chemical stimulus essential to the expression of this growth activity (11, 12).

But the fact that the cells did exhibit what may be taken as tentative steps towards divisions is not without significance. For the fact that SH apparently stimulated a beginning of division; but that this did not go on to completion is pretty good evidence that more than one set of chemical reactions contributes to the accomplishment of one cell becoming two, and that more than one chemical determinant is essential therefor. This is experimental support for the idea long expressed held by the Senior Author that cell division is not the expression of a single chemical process; but is the expression of a succession of interdependent processes (11, 12).

So much for why SH failed to induce cell increase in number in these salivary gland cells. Now to the positive findings and their meaning.

In 1934 it was postulated that since the separation of one cell into two must by necessity involve at least a partial proteolysis of nuclear and cytoplasmic protein, and since SH is a known accelerator of enzymes productive of partial proteolysis, it is reasonable to assume that it is through this function that the compound exerts its stimulating action on cell increase in number (10).

This was clearly an a priori assumption based not on direct observation; but on correlated data alone.

In 1940 a few observations were made on living cells in free-hand sections of grass root-tips. These showed that SH-proteinase did produce lysis of both cyto- and nucleo-plasm (14). And there was a clearing of the nucleus. Such an enlargement and lightening of the nucleus has also been reported by Chalkley[1] in amoebae. While the concordance of nuclear size increase and clarification in amoebae and plant cells with the salivary gland cell reaction to SH is gratifying evidence of the basic nature of the correlation, it is the latter which provides the penultimate experimental demonstration of the validity of the original postulate. For the following reasons.

The marked absolute and relative increase in size of the SH-treated salivary-gland cell nuclei over that of the controls, when coupled with the increase in translucency is indication of dilution and decrease in density of nuclear contents. Such phenomena are usually attributable to imbibition of water through endosmosis. And there is no reason why this interpretation is not valid here as elsewhere.

There might be a few skeptics who would consider the nuclear enlargement as due to growth or constructive substance increase. But there is evidence that the nucleus of these cells is osmotically active. For occasionally it happened that a nucleus would shrink and decrease in size during the course of experiment. While this did sometimes happen in test nuclei as well as in control, it was also true that frequently the nucleus of the control would shrink while that of the test would remain turgid.

Therefore, because of the osmotic potentiality of the nuclei; be-

[1]Statement made at the Gibson Island Conference on *Chemical Growth Promoters*, August 19th, 1942.

cause the nuclear enlargement of the SH-treated cells was as notable in the 15-minute as in the four-hour experiments; because the cell-as-a-whole did not exhibit such enlargement; and because in the majority of cases the nucleus was proportionally larger in the tests than in the controls as shown by the cytoplasmic-nucleus ratio; the conclusion seems pretty well justified not only that there was osmotic change between nucleus and cytosome; but also that the nuclear enlargement of the SH-treated cells was expression of endosmosis and not of growth in the sense of constructive substance increase.

Now the basis of this endosmosis could not be other than an increase in concentration of molecules which were incapable of passing through the semi-permeable nuclear membrane. Such molecules as would be produced by partial proteolysis of nuclear proteins.

But this is not quite the whole story. Evidence is given by Conklin (6) that lytic phenomena are exhibited by the chromatin material as the nucleus prepares for division. And our data show that it was these beginnings which apparently were brought into existence by SH in the nuclei of our salivary-gland cells. In view of all these facts the findings herein reported would seem to justify the belief "that the increased cell multiplication produced by SH derives in large part from the stimulating effect of this naturally occurring chemical group on that partial proteolysis which is an essential preliminary to the division of one cell into two" (14).

A brief comment on stainability is indicated. It was Conklin's experience that as the resting nucleus prepares for division and the larger chromatin granules increase in size, their stainability also increases (6).

According to our experience the nuclear structures of the SH-treated specimens took Janus green to greater degree than did the controls. That reaction is just one more bit of evidence consistent with the idea that SH induced in these cells a transition from a resting state to one in which the beginnings of division were being experienced.

Now Janus green is a redox indicator (16) and SH has marked redox properties of its own (17). The increased stainability of the nuclear structures in the SH-treated cells could therefore come from at least three sources. It could come from direct action of absorbed SH on the vital dye; it could come from a shift in redox equilibrium of nuclear substance induced by SH action thereon; and it could

come from an increase in free SH groups resulting from the partial proteolysis.

Which of these three possibilities is the true cannot be determined from our data. All we can do is to record the observation and leave the solution to later investigation. The problem is worthy of attention since its resolution could well throw light not only on the chemical changes which occur in chromosomes in the early stages of division; but also on the part SH plays therein.

Finally although our data to provide adequate evidence that SH contributes to cell division through its forwarding action on the preliminary partial proteolysis necessary to the progress of this growth activity; it would be good to make the demonstration more elegant by having chemical analyses of nuclear contents with respect to water content, molecular concentration, and the chemical groups liberated as the cell shifts from a state of functioning activity to a state of pre-division. The nuclei of these salivary-gland cells should be good material for such an extension of the inquiry.

E. Summary and Conclusion

Salivary glands were dissected out of living second and third instar *D. melanogaster* larvae and transferred to dilute (1, 4) sea-water. Test cultures contained 1×10^{-7} sulfur as SH in p-thiocresol. Control cultures were made from the same sea-water without SH. The glands were allowed to stay in the solutions for periods ranging from 15 minutes to 4 hours. They were then removed, stained with a 0.05 per cent solution of Janus green B, and studied under high power. To all outward appearances the cells of glands so treated were alive and uninjured.

Exposure to SH produced definite changes in the nuclei of the gland cells; but not in their cytosome.

In general the test nuclei were more translucent and larger than the controls. The cytoplasmic-nucleus diameter ratio was less. This indicates a proportional increase in nuclear dimension. The size increase plus the increase in translucency is taken as evidence of nuclear endosmosis.

The chromatin of the tests exhibited changes which were suggestive of pre-divisional activity. The nucleoli were definitely more diffuse. These changes are taken as evidence of partial proteolysis and that

such is the source of the increase in molecular concentration productive of the endosmosis.

The chromatin of the tests took the vital dye with greater intensity than did that of the controls. This suggests a shift in redox equilibrium such as could result from increase in free SH incident to partial proteolysis.

While SH instigated some degree of pre-divisional activity, the cell-as-a-whole did not go on to complete division and there was no increase in cell number. This failure may be attributed to the high degree of differentiation of these cells and their consequent loss of potentiality for division.

The picture as a whole is direct evidence in support of the belief previously expressed that the forwarding of proliferation by SH derives in large part from the stimulating effect of this naturally occurring chemical group on that partial proteolysis which is an essential preliminary to the division of one cell into two.

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THE DIFFERENTIATION-FORWARDING EFFECT OF L-PROLINE ON EGGS OF *FUCUS**

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A. INTRODUCTION

Although cell specialization is forwarded by l-proline in a wide range of animals (6, 8, 9, 12, 22) the reaction so far observed in plants has been consistent rather than decisive (1, 14, 15).

While this difference could be explained away on the basis of a difference in absorption and utilization, such would be begging the question and should be resorted to only if direct evidence is unobtainable.

An examination of the plant work suggests that the inadequate data could have come from the use of unsuitable test material, inept technic, or failure to establish a proper measure of differentiation as distinct from other growth activities.

Now the decisive evidence from animals was obtained where the pH was on the alkaline side, while the indecisive results in plants came from conditions where the trend was towards acidity.

This raised the idea it might be a good thing to use a marine plant, since such would be cultured in alkaline sea-water.

What was wanted was an unequivocal answer to the simple question: Does l-proline forward cell specialization in plants as it does in animals?

The establishment of the presence or absence of a simple relation is best attained by the use of simple test material.

An acquaintance with the use of *Fucus* eggs by Whitaker (16) for studies in other fields suggested that such might also serve here. The fact that alkaline sea-water is their natural habitat, and the fact

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that sea-water is their natural culture medium, at one stroke removed two possible obstacles to obtaining decision; viz., acidity and artificial salt mixture. Their relative simplicity removed a third. All that remained was to find out if their cell specialization expression is distinct as well as separable from other growth activities, and in such fashion that it can be estimated with reasonable accuracy.

Preliminary studies and the records of previous workers (2, 3, 13, 16-20) showed not only that the changes incident to fertilization and the first cell divisions were clean-cut and decisive, but also that it was possible to mark and measure separately therefrom the incidence of cell specialization as expressed in early rhizoid formation. They were therefore used in the following work.

B. Materials and Methods

The eggs of *Fucus spiralis* (Linn.) were chosen because such are available throughout the summer and the parent alga is abundant on the Provincetown breakwater. The following procedure was developed.

Plants carrying large fruiting tips were collected at early morning low tide. The large tips gave more and better eggs. Early collection tended to stabilize for unequal exposure to drying and water warmed by the summer sun; both of which are factors productive of variability in fertilizability and subsequent growth.

The fronds were brought without submersion to the laboratory immediately after collection in clean white enamel buckets especially reserved for the purpose. They were then rinsed in cold tap-water to get rid of epiphytic marine material and any eggs or sperm which might be present.

After shaking off the excess water the receptacles were snipped off into clean finger-bowls, allowed to partially dry, and put into the refrigerator at 3-5° C. over night. By morning some of the eggs had been extruded together with sperm in the usual gelatinous ooze. Such were placed in 5cm watch-glasses and gently covered with freshly collected filtered sea-water. This broke the antheridian walls and allowed fertilization to take place. The eggs from one conceptacle were usually sufficient in number for an entire experiment. When two had to be used care was taken to distribute them equally between test and control cultures.

The eggs were allowed to remain in the watch-glasses for an hour to ensure fertilizations. Samples were then taken up with a clean glass

pipette reserved for this purpose and transferred to clean hollow-ground glass slides. Test and control samples were taken alternately. A drop of freshly collected filtered sea-water was placed in each slide and the tests were put in one large petrie dish; the controls in another. Both dishes were kept covered and both contained filter-paper wet with sea-water to prevent drying from evaporation.

The gelatinous wall which forms around the egg after fertilization advantageously causes the egg to adhere to the glass. So the preparations were allowed to stand an hour before further manipulation, so that this would occur.

At the end of this time the hollows of the slides were filled. The controls with freshly collected filtered sea-water; the tests with the same water plus l-proline in the desired concentration. Counts were then made of the eggs in each culture and the petrie dishes were set away in the dark. At no time were the cultures exposed to direct sunlight. Culture solutions were changed thrice daily, the last at about 9 P.M. so that the longest stretch without change would be less than 12 hours.

Before going through this procedure in its entirety it was customary to test each batch of eggs for fertilizability by immersion of samples in 2 per cent salt solution. If eggs are fertilized their contents pull away from the new cell wall because of exosmosis. Eggs could be stored for a week and still be fertilized. Sperm did not remain functionable after three days. No material older than 48 hours was used.

All the meticulous details described as necessary for such work were observed, as was scrupulous attention to cleanliness of glassware and avoidance of contamination (4). Test and control cultures were run simultaneously under like conditions of temperature, pH, and illumination.

The l-proline had the same high standard of identity and purity as that of previous studies (8). This was attended to by Drs. Toennies and Lavine of the Philadelphia laboratories. Stock solutions were made fresh for each experiment from freshly collected filtered sea-water. These were kept in the dark in the refrigerator at 3-5° C. No stock solution older than 24 hours was used. Aliquots of the stock were diluted to make the culture solutions with freshly collected filtered sea-water. These were made up fresh for each change.

Five structural states of the eggs were used as indices of growth progression. They are in the order of their appearance:

1. The fertilized unproliferated undifferentiated egg. This is the egg with gelatinous appearing covering wall; but no transverse cell wall or rhizoidal protuberance.

2. The proliferated egg. This is the egg in which two or more cells are distinguishable with or without rhizoidal protuberance.

3. The first-stage differentiated egg. This is the egg in which the rhizoidal bulge and short tapering outgrowth gives evidence of cell specialization beginning.

4. The second-stage differentiated egg. This is the egg in which the rhizoid has become long and slender. It is taken as index of differentiation progression.

5. The super-differentiated egg. This is the egg in which cell specialization goes beyond the usual for the period of observations and produces not one but two rhizoids.

Using these criteria differential counts were made at 18-24; 28-32; and 48 hours after experimental set-up. Such were found adequate for obtaining a picture of the growth reaction of this material to l-proline under the existing conditions. Other conditions might require other spacing. The data in terms of percentage growth expression are recorded in the tables under 22, 30, and 48 hour headings.

Records were therefore had of three consecutive periods of growth activity. Computations were made of the percentage growth expression in each. Using the number of eggs found at each of the developmental states designated above, the following gave the desired measure of the indicated growth activity (Table *A*).

These formulae were applied to the differential counts of each single culture, and to the totals of all the cultures of a given experiment (control and test cultures separately of course). Although there were no significant differences between the two sets of percentages in so far as direction of test difference of test deviation from control was concerned, the tables present the averages of the single culture percentages rather than the percentages derived from the totals, since such are the more valid measures.

A check on the validity of test deviation from control averages is had by comparing the distributions of their respective components. While the Chi square formula could be used, the same end can be attained more simply by arranging the percentages of the components in order of their decreasing value and taking off the amount and direction of test deviation from control under plus, minus, and equal

TABLE *A*

A. *After 22 hours*

1. Proliferation=2+3(+4)/1+2+3(+4)
2. Differentiation (1st stage only)=3(+4)/2+3(+4)
   2nd stage differentiation rare in 1st period; double rhizoids never found.

B. *After 30 hours*

1. Proliferation=2+3+4(+5)/1+2+3+4(+5)
2. Differentiation (1st and 2nd stages)=3+4(+5)/2+3+4(+5)
3. Differentiation (2nd stage only)=4(+5)/2+3+4(+5)
4. Differentiation (2nd stage from 1st)=4(+5)/3+4(+5)
   Double rhizoids rare in 2nd period.

C. *After 48 hours*

1. Proliferation=2+3+4+5/1+2+3+4+5
2. Differentiation (1st and 2nd stages and 2 rhizoids)=3+4+5/2+3+4+5
3. Differentiation (2nd stage and 2 rhizoids)=4+5/2+3+4+5
4. Differentiation (2nd stage and 2 rhizoids from 1st stage)=4+5/3+4+5
5. Differentiation (2 rhizoids)=5/2+3+4+5
6. Differentiation (2 rhizoids from 1st stage)=5/3+4+5
7. Differentiation (2 rhizoids from 2nd stage)=5/4+5

Since growth is a progression concern is with:

a. The percentage of proliferating eggs which went on to express differentiation. A2; B2; C2.
b. The percentage of proliferating eggs which went on to the 2nd stage of differentiation. B3; C3.
c. The percentage of proliferating eggs which produced 2 rhizoids. C5.
d. The percentage of differentiating eggs which went to the 2nd stage. B4; C4.
e. The percentage of differentiating eggs which produced 2 rhizoids. B6.
f. The percentage of eggs at 2nd stage of differentiation which produced 2 rhizoids. C7.

headings. When these are charted in reference to a zero line the consistency with which test values deviate from control in a given direction is easily visualized. Such has been done for these data. The charts are not given because of war-time restrictions on materials. Instead, condensed frequency figures are included in the tables. One set shows the consistency of test deviations among the 14 experiments. Another shows this among the 138 pairs of cultures. There are a few instances where difference between average percentages is in disagreement with the consistency data. An asterisk marks their occurrence, and an appropriate sign after the mark indicates the true direction of deviation. In order to save repetitious exposition in the text, the data from which these corrections are made have been gathered into Table 5.

Now there should be no need to go into extended explanation of the discrepancies. Everybody knows that such may be of common

occurrence in small groups of percentage values where the means may be widely disassociated from the medians by skewed distributions. All that need here be pointed out is that in studies of this kind, frequency of change among a number of cultures is more significant than amount of change in the single cultures, for the latter is conditioned by such variables as pH, temperature, and illumination, while the former is decided only by the single variable, in this case the added l-proline. So then when the difference of averages differs from the consistency of deviation of the components of the averages, the latter and not the former may be taken as the more valid index of direction of change.

C. Results

Table 1 gives the percentage of eggs which expressed proliferation 22, 30, and 48 hours after fertilization. It also gives the number of cultures and eggs obtaining at each of the stated l-proline concentrations. The frequency data are found below the percentage.

The table shows that test proliferation activity was less than control during the first 22 hours of incubation; but that thereafter no significant differences were exhibited. Which is to say that l-proline had an initial retarding influence on cell increase in number.

Table 2 gves the percentage of proliferating eggs which exhibited cell specialization to the 1st stage and beyond. The table shows that while there tended to be slightly less activity in this direction in the tests during the first 22 hours of growth, there was decidedly more by the time 30 hours had passed, and that this was sustained even through to 48 hours. Which is to say that l-proline acted to forward 1st stage differentiation quite definitely after a period of preliminary lagging response.

Table 3 gives the percentage of eggs which went on to the 2nd stage of cell specialization. The first two brackets give the data of this expression in all the growing eggs. The second two give the percentage to which eggs reaching the 1st stage went on to the 2nd. In two of the 14 experiments the 2nd stage was reached during the first 22 hours of incubation. In the remaining 12 this did not show up until 30 hours had passed. The data for the precocious two are given at the bottom of the table.

The table shows that test eggs expressed this advanced stage of differentiation to greater extent than the control at 30 hours, and that

TABLE 1

THE AVERAGE PERCENTAGE PROLIFERATION OF *FUCUS* EGGS IN SEA-WATER CULTURE CONTAINING *l-Proline* AND THE FREQUENCY OF TEST DIRECTION OF DEVIATION FROM CONTROL

M / 1000x	Number Cults		Number Eggs		Percentage proliferation during 22 hours			Percentage proliferation during 30 hours			Percentage proliferation during 48 hours		
x	*C*	*T*	*C*	*T*	*C*	*T*	*T-C*	*C*	*T*	*T-C*	*C*	*T*	*T-C*
500	11	11	345	345	34	30	—4	56	43	—13	73	74	1
500	12	12	278	332	53	62	9	62	68	6	70	79	9
200	8	8	215	198	39	23	—16	60	49	—11	74	53	—21
200	10	10	208	213	25	22	—3	39	46	7	51	51	0
100	10	10	176	184	42	30	—12	59	48	—11	65	61	—4
100	8	8	122	130	22	23	1*−	31	41	10	56	45	—2*=
50	9	9	265	256	43	57	14	55	57	2*=	60	64	4
50	10	10	403	388	40	50	10	89	91	2	93	95	2
20	10	12	329	411	72	65	—7	91	87	—4	92	91	—1*−
20	12	10	616	540	26	20	—6	30	37	"	42	68	26
10	10	10	278	265	57	40	—17	78	69	—9	90	84	—6
5	10	10	591	717	78	75	—3	93	89	—4	94	94	0
1	12	10	616	534	26	36	10	30	41	11	42	74	32
1	10	10	400	502	40	41	1	89	92	3	93	93	0*−
					Frequency Test vs. Control								
					+	−	=	+	−	=	+	−	=
	By Experiments				4	9	1	7	6	1	5	5	4
	By Cultures				56	80	2	65	64	9	70	63	5

C—Control, *T*—Test.

TABLE 2

THE AVERAGE PERCENTAGE EXPRESSION OF 1ST STAGE DIFFERENTIATION OF *Fucus* EGGS IN SEA-WATER CULTURE CONTAINING *l-Proline* AND THE FREQUENCY OF TEST DIRECTION OF DEVIATION FROM CONTROL

M 1000x	Percentage differentiation during 22 hours			30 hours			48 hours		
x	*C*	*T*	*T-C*	*C*	*T*	*T-C*	*C*	*T*	*T-C*
500	81	59	—22	93	83	—10	95	94	—1
500	53	59	6	77	77	0*+	82	91	9
200	72	70	—2*+	96	99	3	97	93	—4
200	27	51	24	72	87	15	92	95	3
100	100	100	0	100	100	0	100	100	0
100	63	47	—16	92	93	1	88	91	3
50	51	31	—20	60	45	—15	70	53	—17
50	91	85	—6	93	100	7	99	99	0*+
20	99	97	—2	99	100	1	99	100	1
20	44	60	16	60	98	38	85	97	12
10	94	92	—2	100	100	0	100	100	0
5	86	87	1*+	91	96	5	95	96	1*+
1	42	72	30	57	97	40	85	99	14
1	92	90	—2	93	100	7	99	100	1*+
	Frequency Test vs. Control								
By:	+	−	=	+	−	=	+	−	=
Exp.	6	7	1	8	2	4	8	3	3
Cult.	46	56	36	65	23	50	50	19	69

C—Control, *T*—Test.

the differential was increased by continued exposure to the amino acid. Thus while test expression at 30 hours was greater than control in 7 of the 14 experiments, less in 4, and equal in 3; at 48 hours it was greater in 11, less in but 3, and equal in none. The same relations held with respect to the progression from the 1st to the 2nd stage. Thus it is evident that l-proline not only stimulated the cells to differentiate, it also acted to advance the progress of their differentiation.

Table 4 gives the percentage of eggs which produced two rhizoids. The first bracket gives the data in terms of all the developing eggs. The second gives the data in terms of all the eggs which exhibited differentiation. And the third gives the percentage to which eggs which reach the 2nd stage went on to produce two instead of one rhizoid. In three of the 14 experiments double rhizoids appeared at 30 hours.

TABLE 3

THE AVERAGE PERCENTAGE EXPRESSION OF 2ND STAGE DIFFERENTIATION OF *Fucus* EGGS IN SEA-WATER CULTURE CONTAINING *l-Proline* AND THE FREQUENCY OF TEST DIRECTION OF DEVIATION FROM CONTROL

M 1000x	Percentage differentiation: Of all developing eggs during 30 hours			Of all developing eggs during 48 hours			Of eggs reaching 1st stage at 30 hours			Of eggs reaching 1st stage at 48 hours		
x	*C*	*T*	*T-C*	*C*	*T*	*T-C*	*C*	*T*	*T-C*	*C*	*T*	*T-C*
500	0	0	0	86	80	—6	0	0	0	90	85	—5
500	0	0	0	73	81	8	0	0	0	88	88	0
200	48	35	—13	73	89	16	49	35	—14	75	95	20
200	16	24	8	70	81	11	22	27	5	77	88	11
100	0	0	0	86	86	0*−	0	0	0	86	86	0
100	0	8	8	60	80	20	0	12	12	68	89	21
50	38	31	—7	59	41	—18	65	66	1*+	84	82	—2
50	9	23	14	76	96	20	10	23	13	77	96	19
20	90	86	—4	94	95	1*+	90	86	—4	94	96	2
20	1	4	3	26	73	47	1	4	3	30	75	45
10	9	5	—4	89	91	2	9	5	—4	89	91	2
5	66	68	2	86	88	2	72	70	—2*=	90	91	1*+
1	1	3	2	26	72	46	1	3	2	30	73	43
1	10	25	15	76	95	19	10	25	15	77	95	18
	Frequency Test vs. Control											
By:	+	−	=	+	−	=	+	−	=	+	−	=
Expts.	7	4	3	11	3	0	7	3	4	10	2	2
Cults.	50	31	57	101	32	5	49	31	58	91	26	21

During 22 hours

M/50,000#1 Cont. 14%; Test 8% M/50,000#1 Cont. 24%; Test 34%
M/5000 Cont. 14%; Test 0% M/5000 Cont. 16%; Test 0%

Frequency

M/50,000#1 3+ : 4− : 2= M/50,000#1 6+ : 0− : 3=
M/5000 0+ : 10− : 0= M/5000 0+ : 10− : 0=

C—Control, *T*—Test.

In the remaining 11 this phenomenon was not expressed until 48 hours. The data of the precocious three are appended at the bottom of the table.

The over all picture shows that the percentage of eggs producing two rhizoids was greater in the test than control cultures. This means that l-proline acted to forward this as well as the other expressions of cell specialization.

TABLE 4

THE AVERAGE PERCENTAGE PRODUCTION OF DOUBLE RHIZOIDS BY *Fucus* EGGS IN SEA-WATER CULTURE CONTAINING *l-Proline* AND THE FREQUENCY OF TEST DIRECTION OF DEVIATION FROM CONTROL

M / 1000x	Percentage of double rhizoids at 48 hours: Of all developing eggs			Of all differentiating eggs			Of eggs which reached 2nd stage		
	C	*T*	*T-C*	*C*	*T*	*T-C*	*C*	*T*	*T-C*
500	12.1	13.4	1.3	12.5	14.1	1.6	13.8	16.2	2.4
500	4.2	5.2	1.0*=	5.2	5.3	0.1*−	5.9	5.7	−0.2*−
200	7.1	8.8	1.7	7.4	9.3	1.9	10.4	9.9	−0.5*+
200	2.1	4.9	2.8	2.7	5.8	3.1	3.1	6.4	3.3
100	4.2	13.6	9.4	4.2	13.6	9.4	4.7	15.4	10.7
100	6.1	19.2	13.1	6.7	20.3	13.6	8.5	22.6	14.3
50	2.4	2.2	−0.2*−	5.8	4.2	−1.6	6.9	5.5	−1.4*=
50	2.0	7.0	5.0	2.0	7.0	5.0	2.5	7.3	4.8
20	5.2	3.7	−1.5	5.2	3.7	−1.5	5.6	3.9	−1.7*+
20	0.6	1.5	0.9	0.7	1.5	0.8	4.4	2.1	−2.3
10	5.9	8.6	2.7	5.9	8.6	2.7	6.6	9.3	2.7
5	1.1	1.7	0.6	1.2	1.8	0.6	1.3	1.9	0.6
1	0.6	3.6	3.0	0.7	3.7	3.0	4.4	5.3	0.9
1	1.9	3.1	1.2	1.9	3.1	1.2	2.5	3.2	0.7
	Frequency Test vs. Control								
By:	+	−	=	+	−	=	+	−	=
Expts.	11	2	1	11	3	0	11	2	1
Cults.	75	24	39	75	24	39	72	25	41
M/1000x	*During 30 hours*								
x	*C*	*T*	*T-C*	*C*	*T*	*T-C*	*C*	*T*	*T-C*
100‡2	0.0	8.1	8.1	0.0	11.6	11.6	0.0	50.0	50.0
20‡1	0.0	2.8	2.8	0.0	2.8	2.8	0.0	3.3	3.3
5	0.1	0.0	−0.1	0.1	0.0	−0.1	0.2	0.0	−0.2
	Frequency								
	+	−	=	+	−	=	+	−	=
100‡2	4	0	4	4	0	4	4	0	4
20‡1	8	0	2	8	0	2	8	0	2
5	0	1	9	0	1	9	0	1	9

C—Control, *T*—Test.

If growth inhibition may be used as an index of toxic influence (11), then there is no evidence that l-proline was toxic to Fucus eggs during a 48 hour exposure to concentrations ranging from M/500,000 to M/1000.

Neither is there any evidence of a concentration reaction gradient save in the matter of double rhizoid production. Here an optimum response at M/100,000 obtained with much less reaction at lower and higher concentrations.

TABLE 5

THE CONSISTENCY OF TEST DEVIATION FROM CONTROL IN SINGLE CULTURES WHERE THE DIRECTION DIFFERS FROM THE AVERAGE PERCENTAGE VALUES OF THE GROUP AS A WHOLE

M/1000x	Hrs.	+	−	=	M/1000x	Hrs.	+	−	=
	Table 1					*Table 3*			
100	22	2	5	1	100 *L*	48	1	8	1
100	48	3	4	1	20 *L*	48	5	3	2
50	30	4	3	2	50 *R*	30	5	3	1
20	48	0	10	0	5 *R*	30	5	4	1
1	48	3	6	1	5 *R*	48	8	1	1
	Table 2					*Table 4*			
500	30	6	4	2	500 *L*	48	3	3	6
200	22	4	2	2	500 *M*	48	2	4	6
50	48	2	0	8	500 *R*	48	2	4	6
5	22	7	2	1	200 *R*	48	4	2	2
5	48	7	0	3	50 *L*	48	0	5	4
1	48	3	0	7	50 *R*	48	2	2	5
					20 *R*	48	4	2	4

L—Left Bracket, *M*—Middle Bracket, *R*—Right Bracket.

D. DISCUSSION

The ability of some plant forms to withstand high concentrations of l-proline without outward evidence of untoward reaction is in sharp contrast to what happens to animals and the plant Ulva (15). The data so far show that l-proline is not toxic to Fucus eggs, and root-tips of cotton, bean (1), and grass (14) seedlings. On the other hand, not only is the plant Ulva adversely affected by concentrations much lower than those found harmless for other plants; but concentrations were found toxic to animals in those cases where wide ranges allowed such estimate to be made (8, 22).

This is no place to go into the possible mechanisms whereby a compound such as l-proline may at one concentration be an effective stimulant to cell specialization, and at high concentrations act to retard all growth expressions. This deservedly interesting topic is laid aside for future exploration. All that will be done here will be to point out that differences between organisms with respect to l-proline toxicity could stem from differences in oxidation processes. Chlorophyll is an active agent in Ulva; and hemoglobin or its equivalent in animals. But such are absent from root-tips, and could be relatively dormant if present in Fucus eggs. Interestingly enough in the latter while double rhizoid production is not inhibited by l-proline, it is much less forwarded in high concentrations than at the apparent

M/100,000 optimum. It may or may not be that this cut in effect is associated with a development of chlorophyll activity; or its equivalent, with growth of the egg. These are merely suggestions and nothing more. The differences are real; the basis of the differences is real; the suggestions concerning the basis may well be unreal.

In this connection attention should be called to the observations of Philip R. White which yield the idea that oxidation processes as related to alkalinity are factors of significance in differentiation expression (21), and for which there is some other indirect evidence (10). It would however require a clairvoyant to predict how this probable interdependence of l-proline, oxidation, alkalinity, cell specialization, and toxicity will be eventually worked out. And our clairvoyant ability is at present in eclipse.

The absence of a concentration reaction gradient was puzzling since such has been exhibited in all previous work, not only with l-proline but also with the other naturally occurring tissue components of general distribution.

Temperature differences did exist as between experiments, and this may have been a factor. But this variable has also been present in the other studies and did not there prevent the differential reaction to concentration change. The resistance to toxic action suggests that intrinsic factors may have limited the amount absorbed; or have acted to do away with non-utilizable excess. Furthermore it is possible that differences in maturity of eggs and/or sperm of the batches used for the separate experiments were a factor. While it would be nice to know the answer, the absence of such does not detract a whit from the finding that l-proline acts to forward cell specialization in plant forms just as it does in animal.

The retardation of cell increase in number during the first 22 hours was a reaction like that obtaining elsewhere (8, 15). It is evident that the amino acid has proliferation retarding properties. This could be due to a direct action on the chemical processes of division. On the other hand it is conceivable that the retardation is a result of a kick towards specialization (5, 6, 7). In other words the progression of the egg from the one cell to the two cell stage could have been delayed because l-proline set the processes of specialization inherent in the former into motion before their time, and not because it acted upon proliferation as such at all. And so on similarly for the other instances.

Recovery from such would be expected since the egg at the single cell stage could presumably be pushed towards specialization just so far and no farther. This because the reaction basis for progressive specialization is not present in the beginning, but must be developed by the chemical changes brought about by successive divisions. While this limitation on the extent to which specialization could be pushed would retard progression from the one to the two cell stage, it would not prevent the progression, and given time the eggs which are held back at first would come to divide because primarily set therefor and the percentage expression could as it does come to equal that of the controls.

How recovery could occur in the continued presence of l-proline if proliferation is held back by direct action of some chemical group provided by the amino acid is hard to see.

On the basis of these considerations inclination therefore is towards the idea that the proliferation reaction to l-proline is not primary; but secondary to the well established specificity of function of the compound in differentiation.

Now although rhizoid production was definitely increased by l-proline after 30 and 48 hours exposure to the amino acid, such reaction did not obtain within the shorter 22-hour period. This latter is not to be taken as evidence of "no-effect" or of inhibition at all. It was simply due to the fact that 22 hours in this material was too short a duration for the cells whose division had been slowed by the antecedent kick towards specialization, to undergo that reorganization essential for progression to rhizoid production. Only that and nothing more. The principles on which this interpretation is based are given elsewhere (7).

These data then and the increase in effect which occurs during the period from 30 to 48 hours of incubation, give unequivocal and clean-cut affirmative answer to the question: Does l-proline forward cell specialization in plants as it does in animals?

This fact, and the fact that a like influence has been demonstrated in a wide range of animal species strengthens the previously stated belief that l-proline provides a naturally occurring chemical group of general distribution which is specific and essential to the enabling of the basic growth activity of differentiation wherever this is expressed.

It may be helpful to others contemplating going into this field of

inquiry to know why the previous work with plant material has been taken as inadequate for sure decision.

Ulva (15) was considered inadequate because no sure measure of cell specialization was afforded. It is true that the apparent lessening of cell increase in number plus the evident increase in cell size of the presumably new cells could be taken as consistent with the idea that a forwarding of differentiation had been had, since similar proliferation reaction had been observed in the presence of l-proline in other instances. But the cell counts and the measurements of cell size by themselves were no proof that differentiation as such had been affected, since the cell increase in size could have come from the opportunity afforded for progressive constructive substance increase by the apparent trend to inhibition of proliferation.

It is also true that cell wall formation can be taken as expression of an intra-cellular specialized process, and from this that the thickening of the walls which seemed to occur in the l-proline cultures was evidence of a forwarding of this particular specialized process. But unfortunately no measurements were made of the occurrence as they should have been. So all the Ulva data really tell is that differentiation may have been forwarded. And while they are consistent with previous work as far as they go, they cannot stand by themselves as incontrovertible evidence that the amino acid forwards differentiation in plants as it does in animals.

Similar and even more stringent obstructions to decision obtained in the work with root-tips of cotton and bean seedlings (1). For here neither cell counts nor measurements of cell size were made. The only data recorded were root-lengths and the distance between root-apex and distal protoxylem. It is no wonder that the author stated: "No clear cut evidence was obtained from either set of experiments to show a specific effect of proline on the rate of protoxylem differentiation." His data could not possibly provide any but the most inferential evidence for or against such a concept.

Now the roots exposed to l-proline did not grow as long as the controls. This of course could be interpreted as coming from the well-established delaying action of the amino acid on proliferation. But even this, without cell counts, would be inferential and quite inadequate for decision that l-proline acts on plant material as on animal.

Furthermore, while the fact that the apex-protoxylem distance was less in l-proline exposed cotton seedlings could be interpreted as due

to stimulation of protoxylem differentiation and thus as consistent with other data, it could also be interpreted as due to delay in cell increase in number.

The picture from the excised bean root-tip cultures where the apex-protoxylem distance tended to be greater in test than control cultures would require still more involved explanation. Thus it is evident that something more than measurements of root growth in length must be had if one wishes to determine whether or not cell specialization is affected in such material. What is needed is not only counts and measurements of cells (7), but also examination of the state of specialization of the protoxylem cells and the numbers thereof.

Recognizing this one of us (F. S. H.) spent some time examining the cleared material left from these experiments. No reliable counts of measurements of the inter-apex-protoxylem space could be made because of maceration. But the distal cells of the protoxylem strands could be easily identified in most cases. Using these as marks of differentiation expression it was pretty generally found that whereas the distal part of the protoxylem strand in the controls was composed of but one or two cells; that of the tests had from three or four. Unfortunately for one reason and another this finding was not adequately prepared for inclusion in the report. But the fact stands that this root-tip material did contain evidence that l-proline had acted to forward cell specialization. The incident is given as an example of how failure to properly establish a measure of differentiation can lead to indeterminate results.

E. Summary and Conclusion

Fourteen experiments were run with close to 10,000 fertilized Fucus eggs approximately half of which were exposed for 48 hours in filtered sea-water cultures to l-proline in concentrations ranging from M/1000 to M/500,000.

Differential counts were made after 22, 30, and 48 hours of the following four types of growth expression, viz., (*a*) those which had proliferated, (*b*) those with short rhizoids, (*c*) those with long rhizoids, (*d*) those with two rhizoids.

There was no evidence of toxic influence. There was no evidence of a concentration reaction gradient save in double rhizoid production.

There was delay in proliferation expression during the first 22 hours

of incubation. This was recovered from by 30 hours and no test difference from control obtained thereafter.

Rhizoid growth and production was taken as the mark of cell specialization. This growth activity was definitely and markedly enhanced in the l-proline cultures. Not only did more eggs produce this evidence of differentiation; but the rhizoids they produced also developed to greater extent. Evidently acceleration was present. Finally the number of eggs where cells specialized to the extent of producing two rhizoids was greater, both absolutely and relatively. In the controls 122 eggs did this; in the tests 198. And the difference was exhibited in 11 of the 14 experiments.

The data justify the unequivocal and clean-cut conclusion that l-proline acts to forward differentiation in plant material as it does in animal.

From this the postulate is strengthened that this naturally occurring amino acid of general distribution provides a chemical group which is specific and essential to the enabling of the basic growth activity of differentiation.

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RELATIVE GROWTH OF THE EUROPEAN EDIBLE CRAB, CANCER PAGURUS: II. GROWTH OF THE ABDOMEN

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A. INTRODUCTION

This is the second of several papers dealing with form changes in the European Edible Crab, *Cancer pagurus*. The first paper (MacKay, 1942) dealt with the relative growth of the carapace. The material presented herein relates to the form changes of the abdomen.

As pointed out at greater length in the earlier report, the study was made at The Laboratory of The Marine Biological Association of the United Kingdom at Plymouth, England, with the aid of a Royal Society of Canada Fellowship in Zoölogy. Inasmuch as the method of collecting the crabs, measuring them, and analyzing the results have been described already in this journal it is not thought necessary to repeat the information here.

It may be of interest to record in this connection the fact that few crabs of this species were found to be parasitized by *Sacculina*, a parasitic crustacean that is often found attached to the abdomens of *Carcinus maenas* and other Brachyurans of the same locality. *Sacculina*, when present, has a marked effect on the growth of the abdomen (Potts, 1910; and Orton, 1936A). Only about 1/10 of 1 per cent of the *Cancer pagurus* crabs examined in this study were found to be so parasitized. Figure 1 shows the ventral side of one of the few parasitized *Cancers* found by the writer. The accumulation of attached marine organisms is evidence that molting has not occurred with the normal frequency. Another parasitized *Cancer* had two *Sacculinas* attached to the abdomen. The behavior of this individual was unusual in that the crab was observed to keep the under side of its body well off the substratum. It moved about on the tips of its legs with its abdomen held high. In all probability contact between the parasitized abdomen and other objects was painful to the animal.

FIGURE 1
A *Cancer paguras* ♂ PARASITIZED BY *Sacculina*

B. How the Measurements Were Made

All of the measurements used in this study were made by the writer and all of the crabs were caught in the vicinity of Plymouth, England. In each case the maximum width of the abdominal segment was taken. Measurements were made with a Glogau sliding jaw caliper calibrated in centimeters.

The parts measured are designated by letters. In the earlier paper (MacKay, 1942) the carapace widths were designated by the letters *A* to *H* inclusive; here the same system is used and the lettering is continued from *I* to *O* inclusive. With the present account 15 body widths extending from the anterior edge of the cephalothorax to the posterior extremity of the abdomen will have been studied in relation

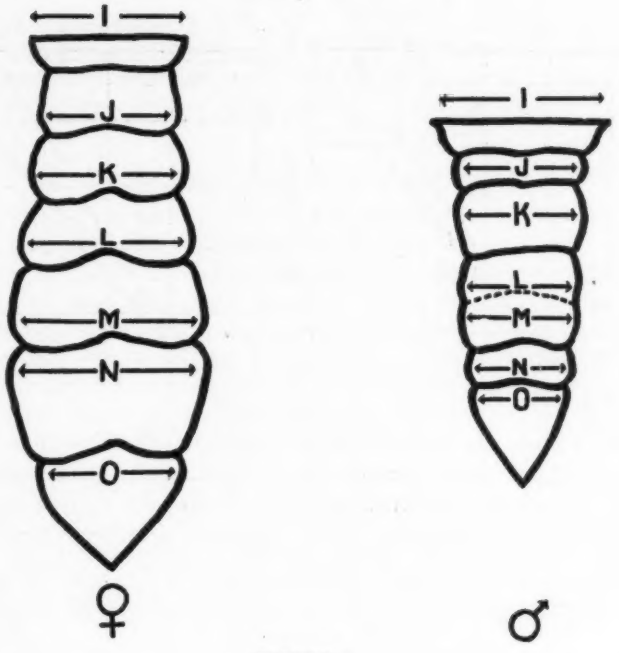

FIGURE 2

DIAGRAMS OF MALE AND FEMALE ABDOMENS SHOWING DIMENSIONS STUDIED
The dotted line on the diagram of the male abdomen separates fused segments.

to carapace length. The following are the abdominal measurements that were studied (see also Figure 2):

1. Abdominal width *I* (the maximum width of the first abdominal segment).
2. Abdominal width *J* (" " " " " second " ").
3. Abdominal width *K* (" " " " " third " ").
4. Abdominal width *L* (" " " " " fourth " ").
5. Abdominal width *M* (" " " " " fifth " ").
6. Abdominal width *N* (" " " " " sixth " ").
7. Abdominal width *O* (" " " " " telson).

The conclusions that follow are based on the analysis of 4643 abdominal measurements, 2342 for males and 2301 for females. The number of measurements for dimensions *I* to *O* respectively for each sex were as follows: 235, 404, 247, 404, 248, 404, and 400 for males

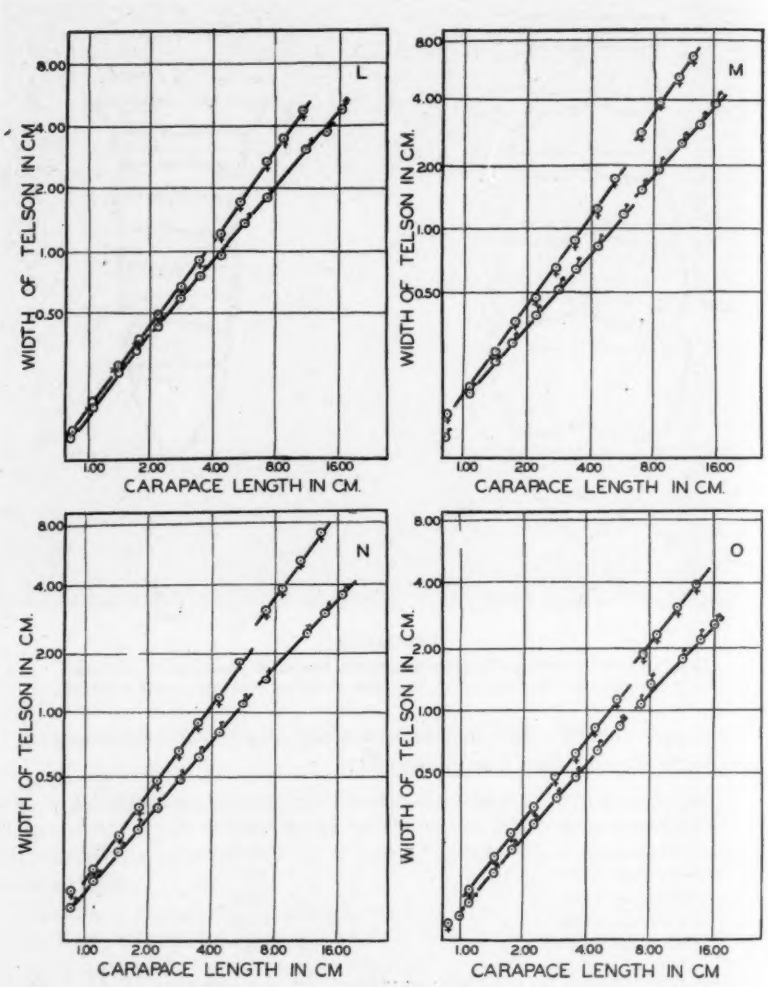

FIGURE 3
GROWTH OF THE ABDOMEN OF *Cancer pagurus*, DIMENSIONS *L*, *M*, *N*, AND *O*

and 245, 384, 257, 385, 257, 387, and 386 for females. A larger number of measurements was available for dimensions *J*, *L*, *N*, and *O* since these were recorded from the first. In order to investigate the gradients of the abdomen dimensions *I*, *K*, and *M* were later added to the study.

C. Relative Growth of the Abdomen

The size of the abdomen in male and female crabs is a secondary sexual character. The abdomen of the male *Cancer pagurus* is narrower than that of the female and the fourth and fifth segments are fused. The pleopods of the first and second segments are modified to form copulatory organs. In the female no fusion of segments occurs, the abdomen is wide, and the pleopods are present on segments two to five inclusive. On extrusion of the ova they become attached to the setae of the endopodites of the pleopods.

The abdominal measurements herein analyzed are graphically represented in Figure 2. In Figure 3 are plotted abdominal measurements *L*, *M*, *N*, and *O;* the data upon which dimensions *L* and *N* are based are given in Table 1. It will be noted that here as in the earlier part of the investigation logarithmic size-classes have been used. The results of plotting all of the abdominal measurements are summarized in Table 2.

D. Relative Growth and Sexual Maturity

Orton (1936B) estimates that *Cancer pagurus* becomes sexually mature at a carapace width of 11.4 to 14 centimeters; this would correspond to a carapace length of 6.7 to 8.3 centimeters. This size, according to the same authority, is probably reached in the third year. These results are in close harmony with those of MacKay and Weymouth (1935) who found that sexual maturity occurs at a carapace width of 10 centimeters in *Cancer magister,* a Pacific coast species belonging to the same genus.

Examination of the results of the analyses summarized in Table 2 will show that the majority of relative growth changes that occur in the abdomen both in the constant k and in the constant b do so at a carapace length of 6.7 to 8.3 centimeters. This, as pointed out earlier, appears to be the size at which members of the species become sexually mature. That changes in the relative growth of parts occur in conjunction with the attainment of sexual maturity has been pointed

TABLE 1

MEANS OF ABDOMEN WIDTHS *L* AND *N* TOGETHER WITH LENGTHS OF CARAPACE OF MALE AND FEMALE CRABS

Lower limits of size classes	No.	Males Mean carapace length	Mean width dimension *L*	No.	Females Mean carapace length	Mean width dimension *L*
0.00	5	0.85	0.13	6	0.88	0.13
1.00	11	1.08	0.18	9	1.10	0.19
1.26	19	1.44	0.26	21	1.45	0.28
1.58	33	1.76	0.33	35	1.81	0.36
2.00	33	2.25	0.43	39	2.25	0.49
2.51	34	2.86	0.58	46	2.84	0.66
3.16	36	3.52	0.75	57	3.51	0.88
3.98	13	4.43	0.93	24	4.39	1.18
5.01	11	5.79	1.35	10	5.53	1.67
6.31	59	7.38	1.77	46	7.43	2.64
7.94	62	8.08	2.16	72	8.71	3.42
10.00	37	11.49	2.95	11	10.78	4.61
12.60	46	13.96	3.65	9	13.50	4.33
15.80	5	16.38	4.62			
Total	404			385		

Lower limits of size classes	No.	Males Mean carapace length	Mean width dimension *N*	No.	Females Mean carapace length	Mean width dimension *N*
0.00	5	0.85	0.11	7	0.86	0.12
1.00	11	1.08	0.15	9	1.10	0.17
1.26	19	1.44	0.21	21	1.45	0.26
1.58	33	1.77	0.27	35	1.81	0.34
2.00	33	2.24	0.34	39	2.25	0.46
2.51	34	2.86	0.46	46	2.84	0.63
3.16	36	3.52	0.59	57	3.51	0.84
3.98	13	4.43	0.77	24	4.38	1.16
5.01	11	5.79	1.06	10	5.52	1.70
6.31	59	7.38	1.38	46	7.43	2.93
7.94	62	8.08	1.68	72	8.71	3.75
10.00	37	11.49	2.28	11	10.79	5.11
12.60	46	13.96	2.84	10	13.43	6.80
15.80	5	16.38	3.48			
Total	404			387		

out by Weymouth and MacKay (1934 and 1936). Similar changes in other Crustaceans, though not so closely related to the species under consideration, have been pointed out by Alcock (1906) for the Indian shrimp *Penaeus;* Kunkel and Robertson (1928) for *Gammarus chevreuxi;* Huxley and Richards (1931) for the shore crab *Carcinus maenas;* Anderson (1931) for *Daphnia magna,* and Marukawa (1933) for the Japanese King Crab, *Paralithodes camtschatica.*

TABLE 2

SUMMARY OF THE RESULTS OBTAINED BY PLOTTING THE VARIOUS ABDOMINAL MEASUREMENTS

Dimensions	Segment	Remarks
I	1	k has a value of 1.09 for females of all sizes and of 1.08 for males of all sizes. There is, however, a distinct difference in the constant b in the two cases.
J	2	For females k has a value of 1.19 until a carapace length of 6-7 centimeters is reached when there is a change to 1.26. Small males approximate the values of k found for small females but later change to a value of 1.07.
K	3	The k for all sizes of males is 1.09 whereas that for females changes from 1.12 to 1.27 at about 2.5 centimeters carapace length.
L	4	In the females k has a value of 1.31 which changes at a carapace length of about 4 centimeters to 1.50. Males change from 1.29 to about 1.17; the change occurs at a carapace length of about 1.5 centimeters. See Table 1 and Figure 3.
M	5	Males of all sizes have a k of about 1.14; there is, however, a change in the constant b at about 7 centimeters carapace length. Females display more marked positive heterogony than the males and have a k of about 1.38 for immature individuals and of 1.40 for mature ones. They also show a change in b at a carapace length of 7 centimeters. See Figure 3.
N	6	Small females have a k of about 1.34 which in larger animals increases to 1.40 with a change in b at a carapace length of 6.5 to 7.5 centimeters. Males have a k of 1.14 until they reach a carapace length of about 8 centimeters after which k decreases to 0.99. See Table 1 and Figure 3.
O	t	For the telson of small females k has a value of 1.24 which at a carapace length of 6-7 centimeters changes to 1.30; this is accompanied by a change in the value of the constant b. In males k changes at 6-8 centimeters from 1.15 to 1.03. See Figure 3.

E. ABDOMINAL GRADIENTS

The growth coefficients presented in Table 2 are graphically shown in Figure 4. It will be noted that the values of k for immature males are almost identical with those for immature females for the first four abdominal segments and that thereafter they differ markedly, particularly in dimensions *M* and *N* (segments 5 and 6). The difference is such that the females and particularly the adult females have a higher differential growth ratio than the males. Since the highest values of k have been found for segments 5 and 6 (dimensions *M* and *N*) it is evident that these segments will make the greatest increases in width. The fact that the abdomens of adult females are

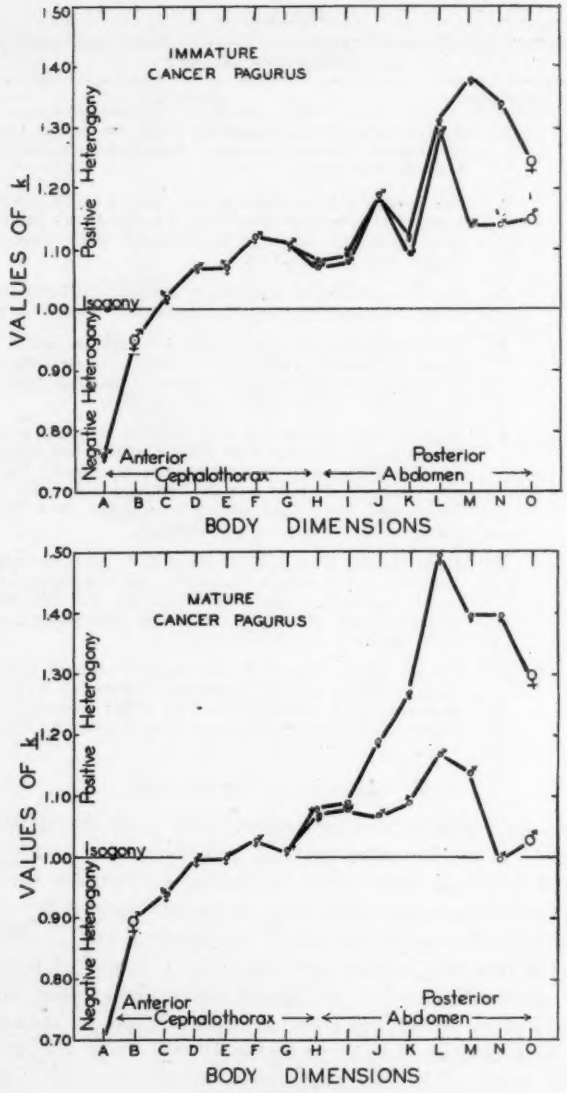

FIGURE 4

GROWTH GRADIENTS OF THE CARAPACE AND ABDOMEN OF *Cancer pagurus*
The differential growth ratios of 15 body widths labelled *A* to *O* inclusive are represented graphically.

much wider than those of adult males probably results from two factors—(*a*) a large increase in the differential growth ratio of females after the onset of sexual maturity, and (*b*) a decrease in the differential growth ratio of males. The inevitable result of the working of these two factors is a female abdomen that will become increasingly wider than that of the male. That a wide abdomen is advantageous to the female for the protection of extruded ova during the long period of attachment is extremely probable; in the male no important function would be served by an equally wide abdomen.

Figure 4 includes the findings for the cephalothorax (MacKay, 1942) as well as those for the abdomen. Both the males and females are represented as well as both young and adult sizes. The differential growth ratios for a total of 15 body widths ranging from the extreme anterior (dimension *A*) to the extreme posterior (dimension *O*) of the animal are included. The figure indicates clearly that the differential growth ratios are higher in the abdomen than in the cephalothorax and that the highest point (the growth center) is in the 4th and 5th segments of the abdomen. The region of most rapid growth is therefore shown to be in the posterior region of the body (k has a value of 1.50 for dimension *L* of adult females) and that the region of least rapid growth is at the extreme anterior end (k has a value of 0.70 for dimension *A*).

F. Sexual Dimorphism

For the eight carapace widths analyzed in the earlier paper only the most posterior one showed any sex difference and that proved to be slight. The abdominal widths, however, do show distinct growth (and consequently form) differences. Other evidences of a similar character will be considered in a later paper in which the growth of the sternum and the appendages will be considered.

G. Summary and Conclusions

In this paper the relative growth of the abdomen of the European Edible Crab, *Cancer pagurus,* is analyzed by the method of Huxley. In an earlier paper in this series (MacKay, 1942) the carapace was studied in a similar manner. An analysis of the widths of the six abdominal segments and the telson in relation to the carapace length based on 4643 measurements indicates a considerable range of values for the constant k. The widths of all abdominal segments display

positive heterogony, k varying between 1.07 for dimension J in males and 1.50 for dimension L in females. This is in the form of a gradient lowest at the anterior end of the abdomen and highest in the middle segments. Considered in conjunction with the carapace gradients for the same species it is apparent that the differential growth ratios are higher in the abdomen than in the cephalothorax and that the growth center is in the middle abdominal segments.

Immature and mature crabs differ in the value of the relative growth constant, the mature individuals having a higher k. Changes in form as indicated by altered values of k and b are correlated with the onset of sexual maturity and probably are causally related thereto. In general females are found to have a higher k for the abdominal segments than the males. After the attainment of sexual maturity this is accentuated by an increase in the differential growth ratio of females and a decrease in males. Females consequently have wider abdomens than males. Very few crabs of this species were found to be parasitized by *Sacculina*.

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Growth, 1943, **7**, 227-238.

CHANGES INDUCED BY SMALL DOSES OF THEELIN AND STILBESTROL ON BODY GROWTH AND ORGAN WEIGHTS OF RATS

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In a recent report, Matthews *et al.* (1942A) described significant changes in body growth and organ weights of female rats after treatment with stilbestrol for periods of 50 and 90 days. It was noted that 50 gamma and 250 gamma of stilbestrol given three times weekly induced similar changes in organ weights, thus indicating that the smaller dose was still considerably above threshold. Even smaller doses (25 gamma) given each five days for a period of 100 days caused degenerative changes in the reproductive organs of young male rats and considerable loss of weight (Matthews *et al*, 1941). Other organs were not included in the report. With these observations as a background, the present study was undertaken in order to study the effects of small doses of stilbestrol on the body growth and organ weights of male rats. The dosage chosen was 1 mg. given in 20 equal doses (50 gamma) at intervals of five days over a 100 day period. This amount of stilbestrol was chosen because it would be in the range of doses frequently given to humans and therefore would not be subject to criticism on the ground that the dose was unphysologic. In addition, for the purpose of comparing estrogens, a similar procedure was used to study the effect of theelin. These data on body growth and organ weights are statistically analyzed and presented for the control, stilbestrol, and theelin treated rats.

A. MATERIALS AND METHODS

Both stilbestrol and theelin were crystalline.[1] These were given intraperitoneally every five days in doses of 0.05 mg. (50 gamma) of crystals dissolved in 0.2 cc. peanut oil. The controls received the oil only. These oil preparations were kept frozen until needed.

[1]Stilbestrol was supplied by Dr. Erwin Schwenk, Schering Corporation, and the theelin by Dr. Oliver Kamm, Parke, Davis and Company.

Albino male rats were carefully chosen; litters were subdivided and weights arranged so that at the start of the experiment the three groups of 25 rats each averaged about 79 grams body weight and were approximately 45 days of age. Body weights were taken at five day intervals and just before the injections were given. The diet consisted of Purina Dog Chow and water. At least three cages of five rats each, one cage for each group, were started the same day and continued as a unit throughout the 100-day test period. These cages were kept at all times in a room having a constant average temperature of 26° C. with a variation of ±2° C.

At the end of the experiment, the rats were anaesthetized with ether, body measurements were taken, the heart was exposed and blood aspirated therefrom as completely as possible by means of a hypodermic syringe. The organs were then carefully dissected free of adherent tissue, placed in closed vials and weighed immediately. Histological material was also saved and fixed in 10 per cent formalin.

B. Results

1. *Growth and Body Weight Observations*

The growth curves of all three groups of rats are shown in Figure 1. It is evident that the curve for stilbestrol is below the curve for theelin and that both curves separated from the controls as early as 20 days after treatment was started. Stilbestrol seemed to have more of an accumulative effect than theelin. This is shown by the spread of the curves near the end of the experiment and by autopsy weights (Table 1). Although the mean body weight of the stilbestrol treated rats was 9.4 per cent below the controls, this difference was not significant. The mean difference divided by the standard error of the difference (subsequently referred to as the "significant ratio") was obtained; if this figure was three or more, the means were considered to be significantly different. The largest "significant ratio" obtained in analysis of body and carcase weights was 2.2 (Table 1). The measurements of body and tail growth are also shown in Table 1. It will be seen that the bodies and tails of the estrone treated groups were somewhat shorter than those of the controls, but these differences are small and all "significant ratios" are below 3. The diameter of the tail root was measured with reasonable care at the hair line by means of callipers. The mean values obtained were 10.3 and 9.6 mm. for the

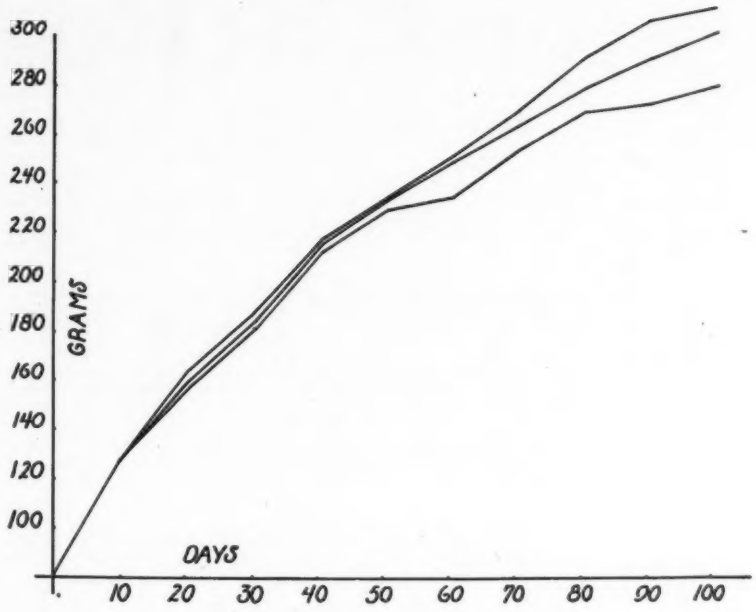

FIGURE 1

GROWTH CURVES OF NORMAL (UPPER), THEELIN TREATED (MIDDLE), AND STILBESTROL TREATED RATS (LOWER CURVE) (25 MALE RATS IN EACH GROUP)

controls and for stilbestrol treated rats respectively; this difference amounts to 6.8 per cent and is significant as shown by the "significant ratio" which is 4.2 (Table 1). Therefore it is concluded that a total dosage of one milligram of either stilbestrol or theelin given over a 100-day period is not sufficiently large to significantly affect body growth of young rats. The diameter of the tail in the stilbestrol treated group seems to be an exception to this.

2. *Weight of Individual Organs*

a. Prostate. The sensitivity of the prostate to small doses of stilbestrol was manifest by a considerable loss in weight (41.1 per cent) and a "significant ratio" of 4.8 before and 4.0 after corrections were made for differences in body weight (Table 1). This histological picture was changed enough to allow selection of these slides from the

TABLE 1

BODY AND CARCASE WEIGHTS (IN GRAMS) OF CONTROL, STILBESTROL, AND THEELIN INJECTED RATS

Measurements in millimeters and organ weights in milligrams. The first line of figures opposite each organ is based on actual weights; under this, organ weights per 100 grams body weight. Twenty-five rats in each of the three groups. Coefficient of Variation $= \frac{SD \times 100}{\text{Mean}}$. Standard Error of Difference $= \sqrt{(SE_1)^2 + (SE_2)^2}$. SE = Standard Error of Mean $= \frac{SD}{\sqrt{N}}$. Significant Ratio = Mean Difference divided by the Standard Error of Difference.

						Significant ratio			Coefficient of variation		
Organs	Controls	Stil-bestrol	Per cent change	Theelin	Per cent change	Controls Stil-bestrol	Controls Theelin	Stil-bestrol Theelin	Controls	Stil-bestrol	Theelin
Body	307	278	−9.4	301	−2.0	2.2	0.5	1.9	15.7	18.6	13.6
Carcase	255	239	−6.3	235	−7.7	1.9	2.2	0.5	11.9	11.7	14.6
Nose anus	226	224	−0.9	224	−0.9	0.5	1.1	—	3.7	4.0	4.8
Tail	172	169	−1.8	169	−1.8	1.0	0.9	—	3.8	3.5	5.5
Nose tail tip	399	394	−1.3	393	−1.6	2.0	2.7	0.3	1.7	3.2	4.3
Root	10.3	9.6	−6.8	9.7	−5.9	4.2	2.6	1.4	2.7	5.2	4.6
Prostate	565	333	−41.1	483	−14.5	4.8	1.9	3.9			
	184	120	−34.8	160	−13.0	4.0	1.7	2.7	27.4	44.1	25.6
Seminal vesicles	1115	631	−43.4	919	−17.6	5.1	2.2	3.7			
	363	227	−37.5	305	−16.0	4.6	2.0	3.2	29.1	42.1	27.4
Epididymides	1004	807	−19.4	932	−7.2	4.5	1.4	1.6			
	327	291	−11.0	310	−5.2	2.4	0.9	0.8	10.5	23.4	30.2
Kidneys	2189	2005	−8.4	2096	−4.2	2.0	0.9	0.9			
	713	721	+1.1	696	−2.4	0.2	0.5	0.7	15.8	17.7	19.2
Spleen	1035	1088	+5.1	988	−4.5	1.0	0.6	1.3			
	337	391	+16.0	328	−2.7	2.7	0.3	2.3	18.4	19.7	34.1
Testes	2694	2441	−9.4	2653	−1.5	2.3	0.5	1.7			
	877	878	—	881	+0.5	—	0.1	0.2	9.7	18.3	12.6
Pituitary	9.4	9.5	—	9.5	—	—	—	—			
	3.1	3.4	+11.1	3.2	+3.3	1.3	0.5	1.1	21.8	22.6	22.4
Thyroid	12.8	13.7	+7.1	13.1	+0.2	1.3	0.3	0.9			
	4.2	4.9	+18.2	4.3	+4.3	2.3	0.7	2.0	22.7	27.0	21.6

TABLE 1 (*cont.*)

Organs	Controls	Stil-bestrol	Per cent change	Theelin	Per cent change	Significant ratio: Controls Stil-bestrol	Significant ratio: Controls Theelin	Significant ratio: Stil-bestrol Theelin	Coefficient of variation: Controls	Coefficient of variation: Stil-bestrol	Coefficient of variation: Theelin
Adrenals	43.3	51.7	+19.4	49.5	+14.3	3.3	2.6	0.9			
	14.1	18.6	+31.9	16.4	+16.3	5.3	3.0	2.6	19.3	18.0	16.2
Brain	1909	1906	—	1931	+1.1	—	0.7	0.8			
	622	686	+10.3	641	+3.0	6.4	2.0	4.1	5.2	5.7	6.0
Heart	1012	961	−5.0	1049	+3.6	1.1	1.3	2.4			
	330	346	+4.8	348	+5.4	1.0	1.3	1.2	17.2	15.3	10.4
Lungs	1680	1503	−10.5	1739	+3.5	2.3	0.3	2.2			
	547	541	−1.1	577	+5.5	0.2	0.7	1.0	23.4	11.7	27.9
Intestines	21.6	24.8	+14.8	27.2	+25.9	2.0	4.3	1.6			
	7.0	8.9	+26.9	9.0	+28.4	4.4	4.6	0.2	20.0	18.6	19.5
Pancreas	1351	1453	+7.6	1544	+15.6	1.2	2.6	1.2			
	440	523	+18.9	513	+16.6	3.0	3.1	0.5	20.9	19.6	16.0
Liver	10909	11378	+4.3	12062	+10.6	0.9	2.3	1.4			
	3553	4092	+15.2	4007	+12.8	3.0	2.8	0.5	17.0	16.4	13.8
Thymus	351	356	+1.4	354	+0.8	0.2	0.1	0.1			
	114	128	+12.3	118	+3.5	1.9	0.5	1.4	25.5	17.7	21.8

normal with consistent results. Some cells were shrunken, others desquamated and degenerated, and the tubule lining as a whole was thin and broken.

Theelin had very little effect on the prostate as shown by a loss of weight of only 14.5 per cent and a "significant ratio" of only 1.9. Thus the prostate of the theelin treated rats was nearly normal. This is further shown by the fact that a significant difference also exists between the weight of the prostate of the stilbestrol and theelin treated rats, the "significant ratio" being 3.9 before and 2.7 after the data were corrected for differences in body weight (Table 1). A histological examination revealed enough change in cell structure so that these slides could usually be distinguished from the controls, but the desquamation was less marked and more cells appeared normal than was found after treatment with stilbestrol.

b. Seminal vesicles. A reduction of 43.4 per cent in the weight of these glands occurred in the stilbestrol treated rats as compared to 17.6 per cent in the theelin group; in only the stilbestrol treated rats was this change significant as shown by a "significant ratio" of 5.1 (Table 1). This difference again illustrates the powerful effect of stilbestrol as compared to theelin. The histology of the seminal vesicles was practically normal in both the stilbestrol and theelin groups.

c. Epididymides. The weights of the epididymides of the stilbestrol treated rats were 19.4 per cent below the control and the "significant ratio" was 4.5. This compares to 7.2 per cent and a "significant ratio" of 1.4 in the theelin treated group (Table 1).

d. Kidney. The kidneys were smaller in both groups treated with estrogens. The weight loss was not significant in either group and when corrections were made for differences in body weight, the means compared favorably with the controls (Table 1). Both an increase (Korenchesrsky *et al.*, 1939; Page *et al.*, 1941; and Matthews *et al.*, 1942A) and a decrease (Frendenberger and Clausen, 1937) have been reported with estrogens given in large doses. A histological examination was made because cloudy swelling of the kidney had been noted in our previous study after treatment with large doses of stilbestrol (Russel *et al.*, 1941). In the present study, cloudy swelling was present in some tubules in all three groups of rats and there seemed to be about as much damage in the controls as in those treated with stilbestrol or theelin.

e. Spleen. The mean weight of the spleen was slightly larger in

the stilbestrol treated group and smaller in those treated with theelin. These differences are without significance. A decrease in weight with larger doses of estrogens has been frequently obtained (Cramer and Horning, 1936; Zondek, 1936; Matthews *et al.*, 1942A). The variability of the spleen is usually considerable (Emery *et al.*, 1940; Matthews *et al.*, 1942A), and this means a large standard deviation, a lower figure for the "significant ratio," and a large coefficient of variation (Table 1).

f. Testes. The testes, although smaller in both the stilbestrol and theelin treated groups, were not significantly changed and after corrections were made for differences in body weights the means were nearly identical in the three groups (Table 1). We had previously found a significant loss in testicular weight after stilbestrol had been given in doses which were even smaller than those used in this study (Matthews *et al.*, 1941).

The histological changes in the testes were slight, some tubules showing signs of broken cells and the number of cell layers was less. Other tubules were normal and as a whole the slides could not be consistently separated from the controls.

g. Pituitary. The weight of the pituitary was not affected by these small doses of stilbestrol and theelin. However, as previously discussed by us (Page *et al.*, 1941; Matthews *et al.*, 1942A), increase in weight has been obtained by many investigators using larger doses of estrogens.

h. Thyroid. The weight of the thyroid was not significantly changed although the data in Table 1 do indicate some increase in weight in the stilbestrol group. This change is in the direction indicated by our previous report (Matthews *et al.*, 1942A).

i. Adrenals. The adrenals of the stilbestrol treated rats were larger by 19.4 than those of the controls and this change was quite significant as indicated by a "significant ratio" of 3.3 before and 5.3 after corrections were made for differences in body weight (Table 1). The increased adrenal weight in the theelin group is not significant. Apparently no one knows what should happen to the adrenals when estrogens are given; the literature is confusing (Matthews *et al.*, 1942A).

j. Brain. The constant weight of the brain was very noticeable in all three groups of rats, but after correction was made for differences in body weight the brains of the stilbestrol treated group were sig-

nificantly larger than those of either the controls or theelin treated rats; the "significant ratios" being 6.4 and 4.1 respectively (Table 1). The coefficient of variation is small (Table 1), conforming data previously reported for the rat (Emery *et al.*, 1940).

k. Heart. The data, after corrections for differences in body weight, show the hearts of the treated rats were slightly heavier but the change appears not to be significant (Table 1).

l. Lungs. Any differences between the three groups are small and not significant (Table 1).

m. Intestines. The weight of the intestinal tract from the diaphragm to the pelvic inlet with the mesentery attached was greater in the estrogenic treated groups, and this increase was especially noted in rats given theelin where it amounted to 26.9 per cent. The significance of the data is also obvious in the theelin group and in the stilbestrol group after correcting for differences in body weight (Table 1). A careful study of food and water intake indicates the increase in intestinal weight was not due to these factors as subsequent discussion will indicate. The increased weight might be due to lack of tonus resulting in a larger accumulative mass of intestinal contents. However, there appears to be no reason why theelin or stilbestrol would depress intestinal tonus. Many tests were made on isolated intestinal segments of rats in Lock's solution. These showed that theelin increased the height of contractions with no noticeable effect on tonus, and stilbestrol had no effect on either contractions or tonus. The insoluble character of stilbestrol in water was considered and therefore stilbestrol was added to the Lock's solution as crystals, in oil solution and in alkaline solution.

n. Pancreas. Although it was found difficult to distinguish pancreatic tissue from the mesentery, this appears to have been accomplished without great variability as the coefficient of variation is relatively small in the three groups of rats (Table 1). Both stilbestrol and theelin increased the weight of the pancreas and these changes became significant after corrections were made for differences in body weight.

c. Liver. The liver was increased in weight by both estrogens and this was more evident after corrections were made for differences in body weight. Nevertheless, these changes are small and judging from our previous discussion not even adequate to establish the direction of change (Matthews *et al.*, 1942A).

Histological examination showed some degenerative changes which were manifest by swelling and degranulation of the cytoplasm but the changes observed in those treated with stilbestrol and theelin were also present in the controls.

p. Thymus. Although the thymus was decreased by larger doses of estrogens (Matthews *et al.*, 1942A), the dosage here employed is obviously without effect (Table 1).

3. *Food and Water*

The composition of the Purina Dog Chow was as follows:

Crude Protein not less than 21.0%
Crude Fat not less than 4.0%
Crude Fibre not more than 6.0%
Nitrogen Free Extract not less than 46.0%
Ingredients: Meat meal, dried skim milk, wheat germ, barley malt, dried beet pulp, corn grits, cereal feed (from corn and wheat), dried raisins, soybean oil meal, molasses, riboflavin supplement, carotene, brewers' dried yeast, vitamin *A* and *D* feeding oil, 1% steamed bone meal, 1% iodized salt.

The food was given in wire meshed containers which were placed in tin pans, so that any particles that fell were caught in the pan. Each morning the weight of food and volume of water remaining in the cage were taken, then 300 grams of unbroken food and 240 cc. of water were given. The water bottles were eight ounces in size with stoppers of rubber and drip tubes of copper. After many test periods had been made, 6 cages of 5 rats each, two cages from each of the three groups, were observed for 40 consecutive days, beginning on the

TABLE 2

MEAN FOOD AND WATER INTAKE BASED ON 400, 24-HOUR TEST PERIODS

Upper figures in each group are actual consumption per rat. Lower figures represent consumption per 100 gram body weight.

		Food in grams			Water in cubic centimeters		
	Body weight in grams	Mean	Significant ratio	Coefficient of variation	Mean	Significant ratio	Coefficient of variation
Control		17.47	—	—	34.47	—	—
	2°0	6.24	—	10.8	12.31	—	15.0
Stilbestrol		17.10	3.0	—	34.67	0.6	—
	261	6.55	6.7	9.6	13.27	7.7	14.5
Theelin		16.83	4.9	—	32.88	4.6	—
	276	6.10	3.1	10.8	11.91	3.1	14.6

sixtieth day of the test. These data are given in Table 2. There are two outstanding features of the table. The first of these is the close agreement of the figures in the three groups. The second feature is that in spite of the small differences in mean food and water consumption in the three groups these changes, as measured by the "significant ratio," are quite significant. On the basis of body weight, the stilbestrol treated rats ate more and drank more than the controls, whereas the theelin group consumed less food and water than the controls. These differences are confusing on the basis that the active agent in both cases was an estrogen, and therefore theelin as well as stilbestrol might be expected to stimulate food and water consumption because both of these estrogens increase basal metabolic rate. Perhaps factors that are not estrogenic are involved.

C. Discussion

The dosage of stilbestrol was 1 mg. administered in amounts equal to 0.01 mg. daily for 100 days. This compares favorably to human doses which vary from 1.0 to 15.0 mg. or more daily (Davis, 1940). During the 100 day test period, the stilbestrol treated rats had a mean body weight of 207 grams; therefore, the average daily dose was 0.05 mg. per kilo. This is comparable to a daily dose range from 0.02 to 0.3 mg. per kilo in humans of approximately 60 kilograms weight.

It is interesting to note that the changes induced by theelin are usually less but in the same direction as those brought about by stilbestrol. This is shown in Table 1 for the following: body weight, diameter of the tail, weight of the prostate, seminal vesicles, epididymides, pituitary, thyroid, adrenals, brain, pancreas, liver, and thymus. The arrangement of the organs in this way is based on the data for 100 grams body weight. This arrangement confirms the changes described under "Results," namely that stilbestrol is more powerful than theelin in producing changes in organ weights. This seems to indicate that stilbestrol is the more toxic of the two.

It is quite obvious that there is no known method to adequately compare identical doses of stilbestrol and theelin. Here the comparison has been on a weight basis and all that is claimed is that the dosage by weight of these two crystalline estrogens was the same. The physiological activity of a given amount of stilbestrol is known to be greater than that of theelin by as much as five times if given in oil

solution (Kieitman and Sieckmann, 1939) or even to 30 times if given subcutaneously in alkaline aqueous solution (Matthews *et al.*, 1942C).

The small doses of stilbestrol and theelin as given in these experiments did not suppress fertility, for although there may have been individuals that were sterile many pregnancies were recorded. However, with larger doses of stilbestrol, sterility was easy to obtain, but even after such treatment recovery was rapid (Matthews *et al.*, 1942B).

In previous sections, mention was made of the histological changes in the testes, prostate, seminal vesicles, kidneys, and liver induced by stilbestrol and theelin. Since the liver has the highest concentration of stilbestrol of all organs (Dingemanse and Tyslowitz, 1941), one might expect the most marked changes in it. Actually, however, the reproductive system is affected most as shown by our previous report (Matthews *et al.*, 1941) in which the testes appeared to be affected more than other organs and by the present report in which the prostate is the only gland that can be consistently separated from the controls by its histological appearance.

D. Summary

Three groups of 25 young male rats were studied for a period of 100 days. All rats were given intraperitoneal injections of peanut oil at intervals of five days for a period of 100 days. In addition, one group received stilbestrol and another group theelin in doses of 0.05 mg. at each injection.

The growth curves of the treated rats as measured by body weight were below the controls, the stilbestrol group being the lowest. Body and tail measurements were similarly affected.

Analysis of organ weights showed that the prostate, seminal vesicles, and epididymides of the stilbestrol treated rats were significantly smaller and the adrenals, brain, intestines, pancreas, and liver were heavier than those of the controls. In the theelin treated rats the changes were less marked; none of the organs was significantly smaller and only the adrenals, intestines, and pancreas were significantly larger than the controls.

The food and water consumption was not greatly different in the three groups, the mean daily consumption being about 6 grams of food and 12.5 cubic centimeters of water per 100 grams body weight.

Theelin increased slightly the height of contraction of isolated in-

testinal segments. Stilbestrol had no effect. Histological studies of the seminal vesicles, testes, kidneys, and liver show that these small doses of stilbestrol and theelin are relatively harmless but that the changes that do occur are more pronounced in stilbestrol treated animals.

The data as a whole show that stilbestrol was more powerful than theelin in bringing about changes in body growth, organ weights, and cellular structure of many tissues. This may be interpreted as arguments for the toxicity of stilbestrol as compared to theelin.

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THE PRENATAL GROWTH OF THE CAT: XIII. THE WEIGHTS OF THE LUNGS, TRACHEA, AND LARYNX IN THE FETAL AND IN THE ADULT CAT

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A recent study by Lassek (1942) of the papers presented at the annual meetings of the American Association of Anatomists from 1912 to 1942, inclusive, shows that only 61, or 1.7 per cent of the 3,655 papers were concerned with the respiratory system. He finds that the respiratory system has received less attention than any other system. However, our knowledge of the structure of the lungs has advanced since the time of Vesalius who in 1543 said, "The substance of the lung is a soft, spongy, thin, light, airy flesh, as though composed of frothy blood or bloody froth, and filled with many branches of vessels" (from Miller, 1937). The anatomy of the adult lung is fairly well known and the development and microscopic structure have received their due share of study, possibly in an attempt to solve the problem of the structure of the lining of the alveoli, a problem which Bremer in 1935 called "over half a century old."

The studies on the quantitative development of the human lung in the postnatal period have been summarized by Wetzel (1934). He gives the weights, volumes, and vital capacities from birth to maturity and the weights of the right and left lungs separately in the adult and also in the postnatal period. Miller (1937), in his exhaustive study of the lung, devotes but a little over one page to the weight, specific gravity, dimensions, and the capacity of the lungs.

The postnatal growth of the rat lung is given by Donaldson (1924), and the compensatory growth of the lung in the rat following surgical removal of a part of the lung tissue has been studied by Cohn (1939). The postnatal growth of the chicken lung has been studied by Latimer (1924) and Mitchell, Card, and Hamilton (1926). The lung weight in the young adult turkey hen has been studied by Latimer and Rosenbaum (1926), and Brody (1941) has reported on the lung weights in

most of the domestic animals and for some of the smaller laboratory animals.

So far as is known the weights of the lungs in the cat have been reported but twice; by Welcker and Brandt (1903) for six adult cats and by Crile and Quiring (1940) for 10 adults.

Materials and Methods

The 229 fetal, 35 newborn, and 104 adults have been described (Latimer and Aikman, 1931; and Latimer, 1936) and these will not be repeated here. The right and left lungs, bronchi, trachea and larynx were removed together, carefully freed of any adherent vessels and fascia and weighed in a glass stoppered weighing bottle on a chemical balance sensitive to 0.1 of a milligram. The smaller specimens were weighed to a tenth of a milligram but the larger fetal, newborn, and adult specimens were weighed to the nearest milligram. As in the preceding papers of this series, the fetal and newborn cats had been preserved in formalin but the lungs, trachea, and larynx of the adult cats were weighed in the fresh condition. Throughout this paper when the "lungs" are mentioned with reference to the fetal, newborn, or adult cats, it will be understood that the weight of the two lungs, the trachea and the larynx is meant. The formulae for the curvilinear growth curves were determined by trial and error as described in an earlier paper (Latimer, 1933) and the rectilinear curves were fitted by the method of least squares (Snedecor, 1938). The formulae used in the determination of the statistical constants for the adult cats are those given by Dunn (1929). The computations were carried to more decimal places than shown in the tables and all of the figures were carefully checked.

Mr. Dale D. Lemon assisted me with most of the routine statistical work on the adult cats and I wish to take this opportunity of expressing my sincere appreciation of his careful, accurate work.

Fetal and Newborn Lungs, Trachea, and Larynx

The increase in weight of the lungs during the fetal period is shown by the line starting at the lower left corner of Figure 1, slightly convex superiorly for the first fifty grams increase in body weight and then continuing as a straight line to 120 grams of body weight, which includes all of the fetal cats. This curve may be distinguished in the figure by the individual weights indicated by circles for the fetal cats and as double circles for the newborn cats. At the time of the begin-

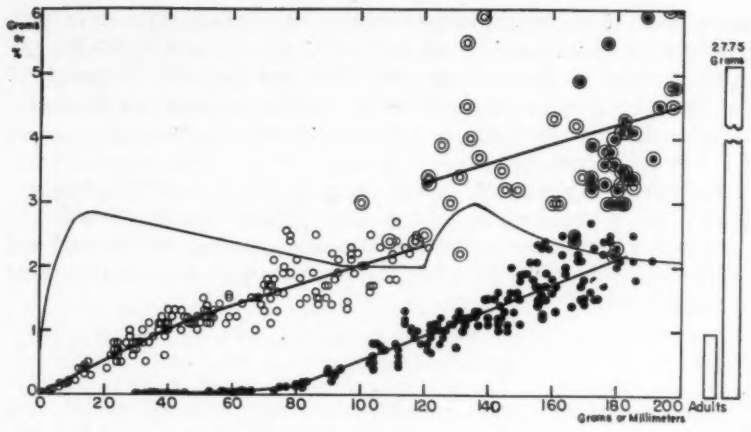

FIGURE 1

THE CURVE STARTING AT THE LOWER LEFT CORNER AND WITH THE INDIVIDUAL CASES SHOWN AS CIRCLES, REPRESENTS THE INCREASE IN WEIGHT OF THE LUNGS, TRACHEA, AND LARYNX, PLOTTED ON BODY WEIGHT

The newborn weights are shown as double circles, and this part of the curve begins at a higher level than that of the oldest fetuses. These same lung weights plotted against body length are shown as dots with the line beginning at 30 mm. of body length. The newborn lung weights are shown as dots enclosed by circles. Empirical formulae for these curves are given in the text. The lighter line without any cases represents the percentage weights of these lungs. The shorter column at the right represents the percentage weight of the adult male lungs and the taller broken column, the weight of the male lungs in grams. The abscissae represent either weight in grams or percentage weight and the ordinates represent either body weight in grams or body length in millimeters.

ning of respiration the lungs increase rapidly in weight and the curve for the newborn cats begins about one gram higher on the figure than the curve for the late fetal cats. The curve for the newborn, like that for the fetal cats from 50 to 120 grams of body weight, is a straight line. The empirical formulae for these curves are as follows:

$Y = 0.1(X^{0.8}) + 0.022X - 0.0984$, from 0.6 to 50 grams, and
$Y = 0.016X + 0.424$, from 50 to 120 grams, and for the newborn cats,
$Y = 0.0145X + 1.582$, from 120 to 200 grams.

In the above formulae, Y represents the weight of the two lungs, the trachea, and the larynx in grams and X represents the body weight in grams. The differences between the calculated and the observed values for each 10 grams of body weight were expressed in percentages of the observed weights and the averages of these percentage devi-

ations are: 10.18 for the first formula, from 0.6 to 50 grams; 4.87 per cent for the second, from 50 to 120 grams; and 11.29 for the third formula for the newborn cats. The average deviation for all of the fetal cats is 7.32 per cent. There was no apparent sex difference in the weights of the lungs in fetal cats and so one formula is given for both males and females.

The same lung weights plotted on body length in millimeters are shown by the curve starting at 30 mm. of body length and with the individual cases shown as dots for the fetal cats and as dots enclosed by circles for the newborn cats. The empirical formulae for this curve are as follows:

$$Y = 0.00003(0.1X)^4 + 0.0007X - 0.0245, \text{ from 30 to 80 mm., and}$$
$$Y = 0.02X - 1.476, \text{ from 80 to 190 mm.}$$

In these formulae, Y again represents the weight of lungs, trachea, and larynx in grams, and X represents the body length measured in millimeters. The average deviation of the calculated from the observed values for the curve based on body length is 8.94 per cent. The deviations for the first formula or for the curve from 30 to 80 mm. of body length is 12.91 per cent and for the formula from 80 to 190 mm. is 5.02 per cent. When plotted on body length or on body weight, the lungs of the newborn cats are not fitted by the formulae for the fetal cats. When these lung weights of the newborn cats are plotted on body length, as shown in the lower curve, the cases are crowded together and so no formulae were developed for the newborn cats. The average weight of the lungs in these newborns was found to be 3.809 grams. In the study of the postnatal growth of the lungs in the albino rat, Cohn (1939) states that ". . . the total lung weight, or any constant anatomical subdivision of the total lung weight, may be considered to bear a straight line relationship to the body weight."

The lighter line without any cases represents the weights of the lungs, trachea, and larynx of these cats as percentages of the body weight and plotted on body weight. This curve shows that in the early fetal period, the lungs increase from a little over half of one per cent to nearly three per cent of the body weight and then from about 15 grams of body weight they decrease in relative weight to about two per cent in the late fetal period. With the beginning of respiration at birth the percentage weights increase to about three per cent. In the adult cats the percentage weights have decreased to about one

per cent. The shorter column at the right in Figure 1 shows the average percentage weight in the adult cats. The percentages are given in Table 1 for the adult lungs. In what part of the postnatal period this decrease takes place we have no information. Scammon (1933) gives similar percentage values for the human lungs for similar periods of development, but his percentages are for the lungs only and these percentages in the cat are for the two lungs, the trachea, and the larynx. Thus the cat lungs are relatively a little lighter than the human lungs.

There is less scatter in the lung weights in the smallest fetuses (Figure 1), more in the larger fetuses, and most in the newborn cats. This may be due to the increased amount of blood in the late fetal, and especially in the newborn lungs, when actual respiration begins and the blood supply to the lungs is increased. Scammon (1923) reports no difference in the weights of the lungs in stillborn and in liveborn human infants. Figure 1 shows very plainly that both the absolute weights, plotted on either body weight or on body length, and the percentage weights are appreciably greater in the newborn kittens. Wetzel (1934) says of the human lungs, "Das lungengewicht des kindes ist vor allem von der Blutfülle abhängig." Spitka (1904) gives lung weights in six criminals who were electrocuted, and he feels that the method of death contracts the thorax and prevents the filling of the lungs with blood. This problem of variability in the weights of the lungs will arise in the discussion of the adult cat lungs later on. In the preparation of the adults, special precautions were taken to see that all blood could drain from the cats. The cats were anesthetized, the external measurements made as rapidly as possible and the head removed, and the body was then suspended to allow the blood to drain out through the large vessels of the neck. Possibly much of this accumulation of blood in the lungs could have been prevented by the injection of some anticoagulant just as the cat was being anesthetized. Unfortunately this was not done and the result is that in some of the specimens the lungs are far above the average weight. In the adults all of the lungs which were obviously filled with blood and redder in color, were marked on the original data cards and these pairs of lungs were omitted in some of the computations for the adult cats (Tables 1 and 3). The lungs of the chicken (Latimer, 1924) were found to contain variable amounts of blood. It would be interesting to see if this variability in lung weight could be prevented by the use of some anticoagulant.

In general, it may be seen that the weights of the lungs increase a

little more rapidly than body weight and a little less rapidly than the body length in the early part of the fetal period, and as an arithmetical function of both body weight and body length in the later part of the fetal period. The lungs are definitely heavier in the newborn than in the late fetal cats. The lungs increase rapidly in percentage weight in the early fetal period and then lose a little after this early growth increase. There is a marked incease in the newborn, in both absolute and in percentage weight.

The ratio of the heart weight to the lung weight (Wetzel, 1934) is given as 1.83 for the human newborn and about 2.00 for the adult. The lung weight was divided by the heart weight in each of the fetal, newborn, and adult cats. These quotients were plotted on body weight and for the fetal cats the curve rose rapidly from 0.5 in the very smallest fetuses to a ratio of 4.0 at 35 grams of body weight, and then decreased slowly to a quotient of 2.5 at the end of the fetal period. In the newborn this formed an average quotient of 3.5. The quotients for the adult males were plotted on body weight and the resulting curve started at 2.8 in the smallest adult cats and decreased, more rapidly at first and then slowly, to about 1.9 in the largest males. The curve for these ratios in the females likewise has its maximum of 2.5 in the smallest adults decreasing to 1.9 in the cats of about 2900 grams and then the tendency seems to be to rise slightly in the heaviest females, or to slightly above a ratio of 2.00.

The relatively small quotient of 0.5 in the smallest fetuses is due to the early development of the heart as well as to the slower initial growth of the lungs. The lungs serve no purpose in fetal life and so they develop later and as they do grow the ratio rises. A comparison of the percentage curve of the lung weights in the fetal period (Figure 1) with the similar percentage curve of the heart weight (Latimer, 1942) shows that the lungs increase rapidly at first while the percentage weight of the heart is decreasing. The heart from 25 to 190 grams of body weight forms a constant precentage of the body weight, with individual variations of course and the curve in Figure 1 shows that the relative weight of the lungs decreases from 15 to 120 grams of body weight. Hence the more marked increase in this ratio in the early period is to be expected and the slow decrease in the later part of the fetal period only follows the drop in the percentage curve of the lung weight. In the adult males the average relative heart weight is constant and in the adult females the heart is slightly heavier in

the medium sized cats, and with the decrease in relative size of the lungs in both sexes in the adult (to be described later) it is but natural that ratio of the heart weight to the lung weight should decrease.

The ratio of heart weight to lung weight in the albino rat was found by using the heart weights and lung weights (both lungs only) as given by Donaldson (1924) for various body weights. In the postnatal growth of the rat, the ratio starts at 2.5, and decreases to 1.4 and this is followed in the largest rats by a slight rise to 1.56. The average ratio of the heart weight to the lung weight for the male cats was 2.13 ± 0.04 and for the females, 2.09 ± 0.03. Unfortunately my data and that of Donaldson for the rat are not fully comparable as the lung weights in the cats included the weights of the trachea and larynx. It would seem that the heart-lung ratios in the rat and in the cat are somewhat alike and that both are lower than that found for man.

Adult Cats

The usual statistical constants for the adult cats are shown in Table 1. The average weights in grams and the coefficients of variation for the 52 males and the 52 females were determined, and these are called "all lungs" in this table. When the lungs were removed and weighed, all lungs which were distinctly red in color or showed evident filling with blood, were marked "bloody" on the autopsy card. A second group which consisted of only the lungs which appeared perfectly normal at the time of autopsy were studied by themselves and the results given in Table 1 as "clear lungs only." There were 37 males and 36 females

TABLE 1

Weights in Grams and Percentage Weights of the Lungs in the Adult Cats

	Males		Females		Difference
	Weight in grams	Coefficient of variation	Weight in grams	Coefficient of variation	*PE* Difference
All lungs	27.75 ± 1.11	45.31 ± 3.56	24.81 ± 0.84	36.07 ± 2.68	2.12
Clear lungs, only	21.91 ± 0.49	20.63 ± 1.66	19.64 ± 0.36	16.42 ± 1.34	3.71
	Percentage weight		Percentage weight		
All lungs	0.987 ± 0.029	31.78 ± 2.30	1.033 ± 0.035	35.79 ± 2.97	1.01
Clear lungs, only	0.809 ± 0.013	14.86 ± 1.19	0.819 ± 0.012	12.61 ± 1.02	0.06

in these last two groups. These two groups of clear lungs probably give a clearer picture of the true values in these cats than the entire

groups, which included the lungs which obviously were filled with blood. These weights were reduced to percentages of the total body weight and averages and coefficients of variation of these percentages are included in the lower part of this table.

The lungs are, on the average, heavier in absolute weight in the males but they form a slightly larger percentage of the body weight in the females. The only statistically significant sex difference is found in the weights in grams of the clear lungs. Naturally the entire groups, male and female, have heavier averages with all of the lungs included, and the percentages likewise are greater.

The coefficients of variation for the absolute and the percentage weights are high, while the coefficients for the clear lungs are about half as great and are fairly good coefficients. The only data giving any measure of variability for human lungs, which are known, are some volumes given by Bach (1926). Unfortunately these are volumes rather than weights and only the averages and the range are given. The variability in volume should be about the same as the variability in the weight of the same organ, but to get coefficients of variability, it is necessary to have more data than are available for these human lungs. It seemed that the range expressed as a percentage of the mean might give some indication of variability by means of which groups of different sized specimens could be compared. The range, or difference between the largest and the smallest, was multiplied by 100 and divided by the mean and the average of these values or the "percentage range" for all groups and for both sexes was 76.0 per cent. Similar values for the clear lung weights in all of my adult cats were 76.8 per cent, or

TABLE 2

EMPIRICAL FORMULAE FOR THE LUNG WEIGHTS IN THE ADULT CATS
(All weights are in grams and all lengths in millimeters.)

Empirical formulae					Average percentage deviation of calculated from observed values
All male lungs	= 0.0063	(body weight)	+	8.234	3.60
Clear male lungs only	= 0.00414	(body weight)	+	10.43	1.86
All male lungs	= 0.193	(body length)	—	72.844	14.41
Clear male lungs only	= 0.104	(body length)	—	32.296	3.18
All female lungs	= 0.0078	(body weight)	+	5.74	9.32
Clear female lungs only	= 0.005	(body weight)	+	7.415	1.40
All female lungs	= 0.126	(body length)	—	39.324	3.97
Clear female lungs only	= 0.092	(body length)	—	27.188	1.00

the variability in weight of the clear cat lungs was much like that of the volumes of the human lungs.

To give a better method of predicting lung weight from either body weight or body length in the adult cats, empirical formulae are given in Table 2. For cats of average weight the averages in Table 1, of course, will adequately predict the weights and percentages, but for cats above or below the average body weight or length, these formulae should give more accurate values. These formulae are valid only within the following limits of body weight: 1700 to 4300 grams for the males and 1500 to 3500 grams for the females. The limits for the body lengths are: 450 to 600 mm. for the male cats and 450 to 560 mm. for the females. All of these curves are straight lines, and as stated above they were fitted by the method of least squares. The last column in this table gives the average percentage deviations of the calculated from the observed values. Since these are simple rectilinear curves it does not seem necessary to take the space to reproduce them here. Naturally there was far more scatter when all of the lungs were included than when only the clear or non-bloody lungs are included. For the latter there was only a moderate amount of scatter (see coefficients of variation, Table 1).

The percentage weights of the male lungs were plotted and the curve, drawn through the medians for each 500 grams increase in body weight, decreased rather rapidly at first from its maximum of 1.07 at 1700 grams of body weight to about 0.7 per cent in the larger cats. These values are for the clear lungs only. For the females, the curve likewise decreased from about 0.9 per cent at 1500 grams of body weight to about 0.7 per cent at 3500 grams of body weight, for the clear lungs only.

In general, the lungs are heavier in the male cats but form larger percentages of the body weight in the females. There is no significant difference in the percentage weights of the male and female lungs and only the absolute weights of the clear lungs are significantly heavier in the male cats. Empirical formulae are given for the more accurate determination of the lung weights in cats of more or less than average body weight or body length. The lungs are relatively heavier in the smaller cats both male and female.

To see how the weights of the lungs vary with the body weight, the body length, and with the weights of some of the other organs in the adult cats, correlations with these dimensions were found. Table 3

TABLE 3

CORRELATIONS WITH THE LUNG WEIGHTS IN THE ADULT CAT

	Correlations with all lung weights		Correlations with the clear lung weights only	
	Males	Females	Males	Females
Body weight	+0.550±0.065	+0.370±0.081	+0.669±0.060	+0.736±0.052
Body length	+0.502±0.070	+0.390±0.079	+0.751±0.048	+0.668±0.062
Integument	+0.600±0.060	+0.227±0.089	+0.653±0.063	+0.732±0.052
Musculature	+0.607±0.060	+0.250±0.091	+0.611±0.070	+0.520±0.086
Skeleton	+0.473±0.074	+0.429±0.079	+0.825±0.036	+0.521±0.086
Digestive tube w.	+0.369±0.081	+0.464±0.073	+0.566±0.074	+0.474±0.087
Digestive tube l.	+0.362±0.081	+0.437±0.076	+0.447±0.088	+0.445±0.090
Liver	+0.532±0.067	+0.391±0.079	+0.498±0.082	+0.506±0.084
Pancreas	+0.330±0.084	+0.378±0.080	+0.308±0.100	+0.641±0.066
Heart	+0.660±0.053	+0.424±0.077	+0.594±0.071	+0.706±0.056
Thyroid	+0.528±0.067	+0.143±0.092	+0.607±0.069	+0.225±0.107
Spleen	+0.495±0.071	+0.191±0.090	+0.363±0.095	+0.327±0.100
Suprarenals	+0.400±0.079	+0.390±0.079	+0.366±0.095	+0.556±0.078
Hypophisis	+0.131±0.093	+0.315±0.085	+0.170±0.108	+0.367±0.097
Kidneys	+0.599±0.060	+0.391±0.079	+0.554±0.076	+0.751±0.049
Bladder	+0.437±0.076	+0.040±0.093	+0.494±0.083	+0.535±0.080
Gonads	+0.516±0.069	+0.317±0.085	+0.444±0.088	+0.395±0.095
Uterus	———	+0.070±0.098	———	+0.348±0.102
Brain	+0.099±0.093	+0.273±0.087	+0.103±0.108	+0.339±0.127
Spinal cord	+0.365±0.081	+0.391±0.079	+0.593±0.071	+0.613±0.070
Eyeballs	+0.358±0.082	+0.273±0.087	+0.451±0.087	+0.665±0.063

shows these correlations with the weights of all of the lungs in the first two columns of this table and similar correlations for only the group of cats having the clear, or bloodless, lungs are given in the last two columns of this table. All of the correlations are positive but several are so low that they are not significant. The average of the 20 correlations for the males are, +0.446 for all of the lungs and +0.503 for the group of cats with the clear lungs. Likewise the 21 correlations for the females are, +0.312 for the entire group and +0.527 for the smaller group of cats with clear lungs. In the second group (clear lungs) the average of the correlations is better in the females, while in the entire group, the males have the higher average. The highest correlation in this table is that with the kidneys in the females in the group of cats with clear lungs, and the lowest in this group is with the brain in the males. This last correlation of +0.103 with a probable error of 0.108 is obviously too low to be significant. There does not seem to be any regularity in the range of correlations in the two sexes. The skeleton, kidneys, body weight, body length, and the integument are found near the top, and the brain and some of the smaller glands are in the lowest group.

The correlations of lung weight with body weight, body length, and with most of the organ weights are not very good when all of the lung weights are used but for the cats with clear or bloodless lungs, the correlations on the average are fairly good. In both groups there is a good deal of variation in the values of the correlations.

Summary

The growth of the lungs, trachea, and larynx in the first part of the fetal period forms a curve convex superiorly when plotted on body weight, and concave superiorly when plotted on body length. Throughout the latter part of the fetal period, and for the adult cats, the weight of the lungs increases as an arithmetical function of either the body weight or the body length. Empirical formulae are given for all of these curves.

The percentage weight of the lungs increases rapidly in the early fetal period to a maximum of nearly three per cent of the body weight and then slowly declines to about two per cent in the late fetal period, then rises abruptly to about three per cent again in the newborn. The adult has a percentage weight of a little less than one per cent.

In the adult cats, the lungs are significantly heavier in the males, but they form a slightly greater percentage of the body weight in the females. There is a marked variability in the weights of all of the lungs, but when a few lungs which were indicated as "bloody" on the original data cards are eliminated, the coefficients of variability are fairly low.

The correlations of the lung weights with body weight, body length, and with the weights of many of the individual organs average +0.503 for the males and +0.527 for the females. With some of the organs the correlations are too low to be significant but most of the correlations are significant.

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Growth, 1943, **7**, 251-252.

THE ABILITY OF HOMOCYSTINE TO SUPPORT RAT GROWTH IN THE ABSENCE OF DIETARY CHOLINE AND METHIONINE

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In this Laboratory young rats have repeatedly been found to grow on methionine and choline-free diets supplemented with homocystine. To investigate this phenomenon female albino rats five weeks old were placed on an amino acid-corn oil diet (1) with eight B-vitamins being administered according to Bennett and Toennies (2). After four days, daily supplements of 18, 36, and 54 mg. dl-homocystine (fed separately in butter) (3) were given to six animals each (Groups *A*, *B*, *C*), resulting in average daily weight gains of 0.6, 1.2, and 1.2 grams, on average basal food consumptions of 2.7, 4.0, and 3.8 grams, per day, respectively.

Replacement of butter by Crisco did not affect the growth responses, nor did omission of the butter and incorporation of 1.25 per cent homocystine in the diet. Omission of homocystine, with butter retained, resulted in immediate loss of weight. Absence of methionine and choline in the food components was analytically confirmed. Observation has yielded no evidence of coprophagy.

In some animals growth continues undiminished after two months, in others it has decreased, and in some an abrupt drop in weight at a rate of three or more grams per day occurs, with basal food consumption declining to zero. One animal of Group *A* began the latter phenomenon on the 39th day of homocystine feeding, one of Group *B* on the 21st day, and three of Group *C* on the 21st, 29th, and 62nd day respectively, suggesting a positive correlation with the homocystine level. Upon addition of choline (3 mg. or more) to the homocystine supplements, eating and growth were resumed after six to nine days of decline; withdrawal of choline after attainment of the former maximum weight resulted in continued growth for 8 to 15 days, climaxed by another drop similar to the former. To date two cases have been

observed where the new decline ended with spontaneous (without choline) resumption of eating and growth, after a fall of about 20 grams.

After six weeks of sustained growth, the B-vitamins of four animals (two of Group *A*, and one each of Groups *B* and *C*) were changed to the modified ryzamin-B, thiamin, riboflavin, nicotinic acid combination of Chandler and du Vigneaud (1). This resulted in changes of average daily growth from 0.7 to 0.3, 0.7 to 0.4, 1.6 to 0.3, and 1.8 to 0.4 grams, respectively. When 40 mg. dl-methionine was substituted for the homocystine in the diet of the first animal, it grew 1.0 grams per day; addition of 18 mg. choline to the diet of the second animal left its growth unchanged. The other two animals were returned to the original vitamin supplement, and growth rates of 1.1 and 1.5 grams per day, respectively, ensued. In two additional animals, receiving 36 mg. homocystine, change from the original B-vitamin supplement to a thiamin, riboflavin, nicotinic acid, pyridoxine combination (1) resulted in changes of growth rate from 0.9 to minus 0.1 grams, and from 1.4 to 0.6 grams, respectively. These rates were increased to 1.1 and 0.8 grams when daily supplements of 18 mg. choline were given.

We are investigating the possible bearing upon our results of the hemorrhagic kidney syndrome of Griffith (4) and of the presumed rôle (5) in bacterial methionine synthesis of p-aminobenzoic acid, which, with its complement (6) inositol, is present in our supplement, while presumably absent in ryzamin (7).

Detailed data, including additional experiments, will be published later.

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BOOK REVIEW

(*Gemma Barzilai. Atlas of Ovarian Tumors. New York: Grune & Stratton, 1943.*)

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This book is bound in loose-leaf style with heavier paper used for full-page illustrations and a lighter paper for text. It begins with a preface by Fred W. Stewart of the Memorial Hospital, New York City, calling attention to the addition of physiological and general biological factors to the descriptive, purely morphological, presentation of ovarian tumors. This is followed by an introduction by the author in which she states that the purpose of the book is to present a "systematic, clear, and complete survey of the complex field of ovarian oncology." The reviewer confirms this statement for it is an excellent presentation. After a general classification of ovarian neoplasms she begins her descriptions of the individual kinds covering 16 varieties. The text, in one sense, is built around the illustrations, a number of which are in color and all of which are of the microscopic appearances which these variegated tumors present, but in the descriptions are included other data which show clearly that the author has the knack of picking wisely from the huge mass of material available the salient points to drive them home. There are brief statistical statements of frequency, references to symptoms, average rates of growth, degrees of malignancy, sizes reached, and so on. Special stress is laid when appropriate, such as in the granulosa cell tumors, the theca cell tumors, the arrhenoblastoma, on the physiological consequences of the growth and activity of these neoplasms upon secondary sex characteristics. Adequate gross descriptions are also included. Descriptions in detail are given of the microphotographs which accompany the discussions of the individual tumors. The photographs themselves are excellent, the fields carefully chosen for characteristic pictures and magnifications of different degrees are judiciously used to bring out the various diagnostic points.

A feature which to the reviewer is quite important is the attention

paid to nomenclature. Having adopted a particular name, the author gives the various other names which have been posed for the same tumor, in some cases amounting to 10 or more, and then states her reasons for adopting the particular one which she uses. Anyone with experience in oncology is only too familiar with the confusion which results from nomenclature alone, and to those teaching the subject it is bedevilment indeed, for often in the same medical school and even in the same clinic various names used for one and the same tumor are a source of never-ending mystification to the students, not to speak of staff members themselves.

Naturally in descriptions as excellent as these, the theme of differential diagnosis passes through all the discussions, a feature of immense importance. Prognosis is given, and final paragraphs concern therapy.

In regard to histogenesis, views embracing embryological relationships are given. Many of these theories may not be of as much importance as the authors of them claim inasmuch as the facts of experimental embryology and other studies of cell behavior make clearer every year that protoplasm is a mighty dynamic system and that fixity is not one of its attributes. To disagree with fixed embryological views and perhaps just in a spirit of cantankerousness, the reviewer usually teaches that a particular tumor doesn't need to arise from a particular embryonal cell, or group of cells, and hence should be named after the cells of origin, but rather that tumors arise from undifferentiated cells and turn into cells which look like particular embryonal cells. This somewhat different point of view has its advantages, not the least of which is the stimulus it gives to further and further studies of cell potency.

In summary then, here is an excellent presentation of the intricacies of ovarian tumors, tersely put, well knit together, logically presented, abundantly illustrated with excellent reproductions in a form in which they should be on the table not only of every pathologist but handy to every gynecologist or general surgeon who operates in the neighborhood in which these tumors appear. The reviewer would go further and recommend it to biologists generally for its presentation of an angle of cell behavior, for if the normal can teach about the abnormal certainly the reverse is also the case. These tumors make fascinating fields of study for the biochemist not only in relation to products which they cause to be excreted, let us say in the urine, but also in

the composition of the tumors themselves, facts which are mentioned in the book. Lastly they are of interest in their bearing on sex modification, for it is highly spectacular indeed to see a woman who has been a competent mother begin to grow a beard and mustache, have her breasts flatten out, and show an aversion to her husband.